Published in final edited form as:
J Toxicol Environ Health A. 2010 January ; 73(15): 1003–1020. doi:10.1080/15287390903566591.

THE ASSOCIATION BETWEEN SEROTONIN TRANSPORTER GENE PROMOTOR POLYMORPHISM (5-HTTLPR) AND ELEMENTAL MERCURY EXPOSURE ON MOOD AND BEHAVIOR IN HUMANS

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Abstract

A functional polymorphism in the serotonin transporter (5-HTT) gene-linked polymorphic region (5-HTTLPR) is reported to affect mood and behavior in humans. In this study, the effects of 5-HTTLPR polymorphism on neurobehavioral and mood domains that are known to be affected by elemental mercury (Hg°) exposure in human subjects were examined. The Behavioral Evaluation for Epidemiologic Studies (BEES) test battery was administered concurrently with urine and buccal-cell collections for 164 male dentists (DD) and 101 female dental assistants (DA) with occupational exposure to Hg° for an average of 19 and 10 yr, respectively. Geometric mean urinary mercury (Hg) levels in DD and DA were 2.52 (2.22) μg/L and 1.98 (1.98) μg/L, respectively. Corresponding indices of chronic occupational Hg° exposure, weighted for historical exposure, were 1212 (1877) and 316 (429). 5-HTTLPR status was 40% and 20% wild type, 40% and 56% single allelic substitution, and 20% and 24% double allelic substitution for the two genders. DD and DA were evaluated separately. Regression analyses controlled for age, premorbid intelligence, frequency of alcohol per week, and education. 5-HTTLPR polymorphism was associated with 5 behavioral measures in DD and with 12 behavioral measures in DA. Mood scores were more consistently associated with the variant in both groups. The strongest evidence for an additive effect for urinary Hg and 5-HTTLPR polymorphism in both groups was for tests of Finger $Tap_{Alternate}$ and Hand $Steadiness_{Factor1}$. Other significant additive effects that were less consistent across groups were also observed. These results add to the growing evidence of genetic determinants of mood and behavior that potentially increase susceptibility to Hg toxicity in humans.

The central nervous system (CNS) is the critical target organ of elemental mercury (Hg°), and evidence from studies of dental professionals suggests statistically significant exposure–effect associations between CNS-related declines and urinary Hg concentrations less than 4 μg/g creatinine (Echeverria et al., 1998; Ritchie et al., 2002; Clarkson, 2002). However, when

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urinary Hg levels approach low concentrations comparable to those observed in the general population (Kingman et al.,1998; Factor-Litvak et al., 2003), it becomes prudent to control for individual factors that may influence sensitivity to Hg-mediated effects, in particular polymorphisms within genes that are known to influence the same neurobehavioral functions that are adversely impacted by Hg° exposure. In this regard, previous studies evaluated polymorphisms of genes influencing the production of coproporphyrinogen oxidase (CPOX) (Woods et al., 2005; Echeverria et al., 2006; Heyer et al., 2006), brain-derived neurotropic factor (BDNF) (Heyer et al., 2004; Echeverria et al., 2005), and the catechol-*O*-methyl transferase (COMT) (Heyer et al., 2009), each of which modifies the neurobehavioral effects of Hg in humans, and have demonstrated increased sensitivity to specific neurobehavioral effects of Hg among subjects carrying such polymorphisms. For example, independent effects of exposure to Hg and the variant for BDNF were observed for the behavioral scores for BEES Finger $\text{Tapping}_{\text{Dominant Hand and Alternating Partialed}}$ in dentists and Hand Steadiness scores and Trailmaking B_{Time} scores in dental assistants (Echeverria et al., 2005). Independent effects of exposure to Hg and the variant for CPOX4 were also observed for the behavioral scores for the BEES Symbol $\text{Digit}_{\text{Rate}}$ in dentists and the BEES Digit $\text{Span}_{\text{Forward}}$ and Beck Depression factor “Worthlessness” in dental assistants (Echeverria et al., 2006). These findings suggest that polymorphisms may affect susceptibility for specific neurobehavioral functions associated with Hg exposure in human subjects.

Of particular note in this regard, several studies implicated an insertion/deletion polymorphism in the promoter region of the serotonin (5-HT) transporter gene (*SLC6A4*) in the development of mood disorders or affective states including anxiety, agitation, and depression (Yoshida et al., 2002; Ito et al., 2002; Gingrich et al., 2003; Caspi et al., 2003; Lin & Tsai, 2004; Zalsman et al., 2006). The serotonin transporter (5-HTT)-gene-linked polymorphic region, termed 5-HTTLPR, has the potential to regulate the transcriptional activity of the 5-HTT gene promoter (Lesch etal., 1996, 1999) and eventually the level of the functional transporter (Kim et al., 2000). In cells homozygous for the long or “l” variant of 5-HTTLPR, serotonin uptake was found to be more than twofold higher compared to cells with one or two copies of the short or “s” variant (Lesch et al., 1996). Therefore, the presynaptic 5-HT transporter likely plays a critical role in sodium-dependent reuptake of 5-HT and has become the main target of most antidepressants (Yoshida et al., 2002; Zanardi et al., 2000; Pollock et al., 2000). Although the variability of the polymorphic repetitive element (5-HTTLPR) is associated with anxiety, depression, and aggression-related traits that influence development of affective spectrum disorders in humans, at least one rodent study (Ansorge et al., 2004) also found that 5-HTTLPR operates more broadly to moderate emotional responsivity to stress.

Because 5-HT acts as a trophic factor modulating developmental processes such as neuronal division, differentiation, migration, and synaptogenesis in the central nervous system, Ansorge et al. (2004) postulated that genetic inhibition of 5-HTT function may be explained by events occurring during early brain maturation and proposed to mimic the effect of genetic *5-HTT* disruption by inhibiting 5-HTT function with the use of the SSRI fluoxetine (FLX). In comparison to saline administration, postnatal treatment with FLX decreased exploratory neurobehavior in both *5-H7T*$^{+/+}$ and *5-HTT*$^{+/-}$ mice pups, as demonstrated by a reduction in the total distance traveled, time spent ambulating, and rearing in the open field, as well as reducing the total number of arm entries in the elevated plusmaze. These findings indicate a critical role for serotonin during early development of the CNS, supporting the idea that genetic polymorphisms that reduce 5-HTT expression may exert their effects by altering maturation of circuits that modulate emotional responses to novelty and stress. This hypothesis provides a potential explanation for increased susceptibility of humans carrying one or two low-expressing *5-HTT* alleles for depression as well as reduced performance on neurobehavioral tests.

The present study addressed the question as to whether subgroups of humans with the 5-HTTLPR polymorphism may be at greater risk for Hg°-mediated CNS effects at comparable exposure levels. Previous studies reported that exposure to low levels of Hg° adversely alters mood expressed as affective symptomology and measured by scores for the Profile of Mood States (POMS) (McNair et al., 1991), the Beck Depression Index (Beck, 1998), and SCL90 (Derogatis, 1977), as well as behavioral domains that encompass attention/short-term memory, working memory, visuomotor processing, cognitive flexibility, and motor function (Echeverria et al., 2005, 2006). Here, the potential adverse exposure–effect associations between exposure to low levels of Hg° and performance scores fur neurobehavioral tests and affective symptomology were examined, where it is postulated that 5-HTTLPR might moderate the associations for both outcomes when exposed to Hg.

MATERIALS AND METHODS

The Study Population

Between 1998 and 2001, 193 male dentists and 230 female dental assistants were recruited for a study of the effects of elemental Hg on the central nervous system. Dentists were selected from among 2675 dentists practicing in Washington State who responded to a short survey questionnaire and returned a urine sample for Hg analysis. In total, 1488 dentists met the study criteria, which included (1) participation in an uninterrupted full-time dental practice for 5 consecutive years immediately prior to enrollment; (2) absence of health conditions that might alter urinary Hg levels, including, but not limited to, kidney disease (e.g., lithiasis, pyelonephritis, orthostatic proteinuria), endocrine disorders, and cancer; and (3) no history of chelation therapy. Eligibility was further restricted to male dentists due to the small number of female respondents.

To ensure a distribution of exposures, potential participants were recruited from this pool of dentists on a stratified random basis, with strata based upon intensity of exposure to elemental Hg as determined by our screening urinary Hg level. Female dental assistants subsequently were recruited from the practices of participating dentists based on the premise that intensity of mercury vapor exposure is strongly associated with office practices and parameters. This selection process thereby stratified dental assistants across levels of Hg exposure comparable to those of participating dentists. These populations were described in greater detail elsewhere (Echeverria et al., 2005, 2006; Heyer et al., 2004,). The institutional review boards of Battelle and the University of Washington approved the study protocol. All subjects provided written consent prior to participating in accordance with the Declaration of Geneva of the World Medical Assembly.

Test Procedures

Study participants were assessed at a central location. All procedures were performed during the single subject visit. Participating subjects took a breath alcohol test prior to additional testing and provided both blood (approximately 2 ml) and spot urine (approximately 50 ml) samples. Buccal cells were harvested from the inner cheek of each subject to provide DNA for genetic testing, as described elsewhere (Woods et al., 2005). Blood samples were collected to measure and control for potential organic Hg and lead exposure. Urine samples were collected to measure total mercury content, as described later in this report. Project staff members who assessed the participants were blinded to all exposure and genetic outcomes. Examiners were also trained and certified by an independent quality assurance team led by Kent Anger and Diane Rholman (Oregon Health Sciences) that included supervised practice sessions with the cooperation of proxy participants to achieve less than 10% variance in test–retest performance and compliance, with standardized scripts and instruction provided to each tester in a written manual for each examination procedure.

Subjects then completed the Neuroquest computerized questionnaire and the Behavioral Evaluation for Epidemiologic Studies (BEES) test battery (Echeverria et al., 2002). The instruments are extensively described elsewhere (Echeverria et al., 2005, 2006; Heyer etal., 2004). The BEES test battery was designed to evaluate behavioral domains with sufficient redundancy and sensitivity to examine subtle Hg effects. For this study, tests were specifically selected for (1) sensitivity to Hg exposure (Echeverria et al., 1998, 2005, 2006), (2) statistical properties (Echeverria et al., 2002), and (3) compliance with recommendations by the World Health Organization (1986) and the Agency for Toxic Substances and Disease Registry (Amler et al., 1995). The BEES test battery was administered using a touch-screen computer and included the following tests: attention (BEES Digit and Spatial $Span_{Forward}$, BEES Trailmaking A); working memory (BEES Digit and Spatial $Span_{Backward}$); sustained attention (BEES Vigilance); visual memory (BEES Pattern Memory); perception (BEES Pattern Discrimination); visuomotor speed (BEES Symbol-Digit Substitution); cognitive flexibility (BEES Trailmaking B); reaction time (BEES Simple and Choice Reaction Time); response speed (BEES Finger Tapping); and tracking (BEES Adaptive Tracking). The BEES behavioral test scores were transformed as previously described (Echeverria et al., 2002, 2005). However, for the BEES Finger Tapping, traditional test scores (Echeverria et al., 2005) were supplemented with a derived score that more accurately measures the cognitive resources needed to coordinate alternate finger tapping. Finger $Tapping_{Alternate\ Partial}$ was derived by covarying out the contribution of the dominant and nondominant finger speed. By eliminating the motor component from either hand, one can now more accurately assess the decision to alternate finger taps. The BEES battery was supplemented with additional standardized tests and control tests to strengthen our interpretation of results. They include:

Visual memory (Wechsler Memory Scale, Visual Reproduction subtest)—The Wechsler Memory Scale (WMS) visual reproduction test (Wechsler, 1945) is a test of memory for nonverbal stimuli. Four line drawings are presented one at a time for a 10-s exposure period. After the drawing is removed, the subject is asked to immediately draw the figure from memory. Performance is scored according to standardized criteria (Wechsler, 1945). The raw score is the dependent variable.

Manual Dexterity (Hand Motor Steadiness Battery, 2000)—This test measures intentional hand steadiness. The task requires participants to hold a pointer for 15 s at the center of a series of holes with decreasing diameters, with the instruction not to touch the sides of the hole. Scores are number of hits and cumulative contact time for each of the seven holes. Time: 3 min.

Hold tests that are not usually affected by mild brain insults are useful covariates in analytic models to control for variation in pre-morbid intelligence. Several tests were examined, including the Reading test of the Wide Range Achievement Test 3 (WRAT-3) (Wilkinson, 1993), the BEES Vocabulary test (Echeverria et al., 2002), and the Test of Nonverbal lntelligence-3 (TONI-3) (Brown et al., 1997). In analytic models, vocabulary scores were not expected to be affected by low Hg° exposure and were used as an index of stable CNS function (Letz, 1993; White & Proctor, 1992). Score was the number correct. Time: 4 min.

A visual acuity test was employed to control for individual differences in vision that might impact some tests. The Snellen Test of Visual Acuity (Optec 2000/25000 Vision Testing System, 1998) is based on character recognition using a standardized chart consisting of lines of letters. The letter on the top line is the largest; those on the bottom line are the smallest. To test one's ability to see at far distances, the participant is positioned with the forehead pressed against the viewer and reads aloud the largest to the smallest line of letters he/she can see until he/she cannot correctly identify the letters. The right and left eyes are tested separately. Scoring

was done on a line-by-line basis. A line was considered read if more than half of the letters (i.e., 4 of 5) are identified correctly.

Vibration sensitivity was used as an indicator of large-fiber peripheral nerve function. Threshold sensitivities were measured using the Vibratron II (1998). Using a two-alternative forced-choice paradigm, the device quantifies detection of vibratory stimuli for the left and right index finger and toe. For each trial the subject is required to determine which of two rods is vibrating. The intensity sequence is determined by an adaptive algorithm. The intensity of the stimulus is reduced by 10% at each trial until participants cannot detect vibration. When the participant makes the first error, the intensity is increased by 10%. These steps continue, with correct trials resulting in a lowering of intensity and errors resulting in an increasing intensity until five errors have been made. The vibration threshold is determined by identifying the five errors and the five lowest correct scores, eliminating the highest and the lowest, and calculating the mean of the remaining eight scores.

The Neuroquest computerized questionnaire collects information on demographic and personal habits, medical histories, work histories, a symptom checklist (45 symptoms) expanded from the Q16 (Smargiassi et al., 1998), and computerized versions of the Profile of Mood States (POMS) (McNair et al., 1991), the Beck Depression Index (Beck, 1998), and the Symptom Checklist-90 (SCL-90) (Derogatis, 1977). Histories of medical conditions are grouped into categories, including physical injury, major operations, digestive, circulatory, sensory, renal, endocrine, immune, nervous system, and emotional problems. Within each category, the use of medications is indicated.

Urinary Mercury Analyses

Urine samples were divided into aliquots for Hg and creatinine determinations. Analysis of total Hg was performed by continuous-flow, cold-vapor spectrofluorometry, as previously described (Pingree et al., 2001). Urinary creatinine concentrations were measured using a standard colorimetric procedure (Sigma number 555-A). Urinary Hg levels were calculated as both micrograms per liter and micrograms per gram creatinine. No significant differences between the two measures were observed. The natural log of the urinary Hg concentration (In μg/L) is used in the present analyses, as this more accurately reflects biological mechanisms (Clarkson, 2002).

Chronic and Peak Mercury Exposure Indices

Chronic Hg° exposure was calculated separately for dentists and dental assistants by taking the product of (1) the mean number of amalgam placements and removals per week, (2) a weighting for the type of amalgam used (1 for pre-encapsulated amalgams, and 2 for office-mixed), (3) a weighting for the time period of the job (1, ≥1992; 1.5, 1985–1992; 1.75, ≥1972 to 1982; 2.0, ≤1970), and (4) the duration of the job. The subject's chronic exposure index was calculated by taking the square root of the sum of these calculations across all jobs in order to normalize the distribution of exposures. Finally, age was covaried out of this index to remove expected collinearity, allowing both variables to be used simultaneously in regression models.

A peak index was also calculated for each subject by multiplying the maximum number of placements and removals by the amalgam type and time-period weightings for each job, and selecting the highest value. The weighting systems used in these calculations were derived from measurements of urinary Hg levels among dental professionals since 1975 (Naleway et al., 1985) and from expert industrial hygiene opinion.

5-HTTLPR Genotyping

Genotyping was performed by the Functional Genomics Laboratory of the Center for Ecogenetics and Environmental Health at the University of Washington and was successfully completed for 164 male dentists (DD) and 101 female dental assistants (DA) from the 194 DD and 233 DA described in previous reports (Echeverria et al., 2006). The procedure employed a polymerase chain reaction (PCR)-based assay to identify the "long" and "short" polymorphic alleles that are located in the polymorphic region of the 5-HTT gene, which consists of the insertion/deletion of 44 base pairs. The PCR primers were as follows: sense strand 5′-ggCgTTgCCgCTCTgAATgC-3′ and antisense strand 5′-gAgggACTgAgCTggACAACCAC-3′. The PCR products were resolved on an ethidium bromide-stained 3% agarose gel, sized using DNA molecular weight markers, visualized with ultraviolet (UV) light, and the alleles were identified as previously described (Woods et al., 2005; Heyer et al., 2004). 5-HTTLPR alleles were classified as long or "I" type if there were 16 sequence repetitions and as short or "s" type if there were only 14 repetitions. Samples were considered wild-type (in their natural form) if they had two "I" alleles (I/I), heterozygous if they had one "I" and one "s" allele (l/s), and "full mutation" if they contained two "s" alleles (s/s).

Statistical Analysis

This study simultaneously examined potential effects of low level Hg° exposure and the 5-HTTLPR polymorphism in the same regression model on affective symptomology and neurobehavioral test scores. It was postulated that 5-HTTLPR, which is known to be associated with increased depression and anxiety, could operate more broadly to adversely affect test scores, particularly when exposed to Hg°. Hypotheses were tested based upon evidence of a "linear exposure–effect" relationship using a fixed regression model. Statistically significant interactions were assessed by the product of urinary Hg and the 5-HTTLPR polymorphism where the heterozygous and mutant subjects were pooled and coded (0/1) in a single variable to avoid small numbers. The fixed model simultaneously evaluated the main effects for urinary Hg concentration, the 5-HTTLPR variant, their interaction term (HgU*5-HTTLPR), age, alcohol consumption (number of drinks per week), premorbid intelligence (BEES vocabulary score), and education (highest level achieved). The fixed model for dentists excluded education, as this was a constant (all dentists achieved the highest level on our scale). The model controls for factors that are known to affect behavior (White & Proctor, 1992; Anger et al., 1997). Secondary analyses for hand steadiness tasks employed an expanded fixed model that included a dichotomous variable to indicate a history of repetitive trauma that might alter performance on hand coordination tasks.

The partial correlation and *p* values for all associations between outcomes measures and urinary Hg concentration, 5-HTTLPR polymorphism, and their interaction term are reported for *p* values <.05. Only associations with *p* values <.05 are considered to be significant indicators of possible adverse behavioral effects. The magnitude of statistically significant effects uniquely attributable to the polymorphism in the presence of exposure to Hg is expressed by the model coefficient for the genetic variant (a dummy variable that calculates the difference in performance between the wild-type and variant group). The proportional increase above that of Hg exposure is also defined as the coefficient divided by the mean performance score of the wild-type or referent group adjusted for other factors in the model.

The analyses also addressed concerns about unwanted alpha error. Bonferroni corrections do not take into account the functional groupings of human performance and ignores even consistent trends across scores when they do not meet significance. To address this problem, hypotheses were tested on two levels. First, a priori effects (those specifically known to be sensitive to Hg) were listed and compared to observed effects. Second, directionality of all

associations within behavioral domains were evaluated for p values <.1 for consistency of directionality in the expected direction (declining performance with increased exposure).

RESULTS

Univariate Comparisons Among Dentists and Dental Assistants

Demographic, health, and genetic distributions for the two study groups are provided in Table 1. Male dentists (DD) were analyzed separately from female dental assistants (DA), a decision supported by observed differences between the two gender groups for many socioeconomic variables that are known to influence performance on behavioral tasks. These variables include age, income, education level, the BEES Vocabulary, the WRAT-3 Reading test, and alcohol consumption. The control tests were not related to urinary Hg. Visual acuity was similar for both groups. Differences in the frequency of the "s" allele among ethnic groups (significantly increased in Asians) have been reported (Chong et al., 2000;Baca-Garcia et al., 2002), and this is, in fact, the observation in the present study population (Heyer et al., 2008). However, the number of non-Caucasians in the present study was too small to affect the overall findings, as confirmed by the observation that adjustment for race did not affect the findings reported herein.

Expected differences in the use of prescription drugs including antidepressants were observed between DD and DA. However, behavioral tests were not consistently affected by medications. Measures estimating occupational exposures to Hg° are presented in Table 1. The contribution of Hg from personal dental amalgam accounts for 0.33 of the amount in urine, comparable to previous estimates (Echeverria et al., 1998). Based on current and chronic exposure levels, DD incur greater occupational exposure than DA. In contrast, personal dental amalgam exposure was comparable in both groups. In addition, expression of variant forms of 5-HTTLPR (l/s + s/s) was more prevalent among DAs (80 versus 60%, respectively).

Regression Analyses

Table 2 presents the means (SD) of behavioral test measures by domain, whereas multiple regression model coefficients for urinary Hg and 5-HTTLPR polymorphism that had a statistically significant relationship at $p < .05$ are presented in Tables 3a and 3b for DD and DA, respectively. For measures that had p values <.05 for both urinary Hg and the genetic variant, the magnitude of the genetic effect and the additional fold increase in decline are presented.

With respect to Hg exposure, the distribution of behavioral findings was similar but not identical for DD and DA. Measures in 9 domains for DD and 8 domains for DA out of 10 domains achieved statistical significance, although the distributions of adversely affected measures were not identical.

No marked associations with any measure of chronic Hg exposure were found. Moreover, for all models that simultaneously evaluated urinary Hg, the polymorphism, and their potential interaction in the same model, no multiplicative interactions between Hg exposure and 5-HTTLPR were observed. However, among DD, two performance measures within the domain of Cognitive Flexibility (for the BEES $\text{Switching}_{\text{Latency (msecs)}}$ and Alternate Finger $\text{Tapping}_{\text{Partialed}}$) and three measures in the domain of Manual Coordination Skills (for the BEES Finger $\text{Tapping}_{\text{Dom//Non-dom Hand}}$ and $_{\text{Alternate Partial}}$ and Hand $\text{Steadiness}_{\text{Factor1}}$) were significantly associated with both urinary Hg and 5-HTTLPR. Among these measures, the greatest increase in decline uniquely attributable to the genetic variant was 1.1-fold for Hand $\text{Steadiness}_{\text{Factor1}}$, whereas the genetic contribution among other scores ranged from 0.04- to 0.15-fold. These results indicated independent modes of action. The contribution in decline

from Hg° exposure and the additional contribution in decline from the polymorphism, when occurring simultaneously, increased overall adverse performance scores.

Among DA (Table 3b) the pattern of significant joint associations between urinary Hg and the 5-HTTLPR polymorphism was distinct from that among DD (Table 3a). Performance on the BEES Digit $\text{Span}_{\text{Forward N Digits}}$, BEES $\text{Vigilance}_{\text{Hits}}$, BEES Pattern $\text{Discrimination}_{\text{Latency (secs)}}$, and three measures within the domain of Manual Coordination (the BEES Finger $\text{Tap}_{\text{Alternate}}$, the Hand $\text{Steadiness}_{\text{Factor1}}$, and BEES Adaptive $\text{Tracking}_{\text{Score}}$) were jointly affected. Comparable to that among DD, the greatest decline uniquely attributable to the variant was two-fold or 1.99 for the Hand $\text{Steadiness}_{\text{Factor1}}$ score and 0.55 for Pattern $\text{Discrimination}_{\text{Latency (secs)}}$ score, whereas the genetic contribution in decline among other performance scores ranged from 0.01- to 0.15-fold.

All other observed significant associations with performance were either with urinary Hg or 5-HTTLPR polymorphism alone. Among DD, one additional single association with 5-HTTLPR variant alone was found for BEES Pattern $\text{Discrimination}_{\text{N Correct}}$. In Contrast, 11 single associations with 5-HTTLPR alone were found among DA with some consistency within the domains of Working Memory and Cognitive Flexibility.

In the Sensory Domain, the test for threshold sensitivity to vibration demonstrated a statistically significant association with urinary Hg in DD and DA, but once control for repetitive trauma was added to the model, statistically significance for the association among DD was lost.

Tables 2 and 4 summarize results for Affective Symptomology. The a priori hypothesis was that exposure to Hg° and the 5-HTTLPR polymorphism is associated with adverse changes in mood states as measured by subscores for the POMS, BDI, and the SCL-90 scales. It was also expected that the prevalence of reporting symptoms was more pronounced among DA. However, it was observed that potential symptoms associated with the 5-HTTLPR polymorphism in both genders were far more prevalent than potential associations with exposure to Hg°. In addition, the evidence of a potential additive effect between urinary Hg and 5-HTTLPR in DD is restricted to the POMS Scale for Confusion and the SCL-90 Factor2. In contrast, the potential evidence for additive effects in DA was distributed across all three instruments, i.e., four out of six POMS scales, two out of four BDI factors, and one out of four Scl90 factors.

Finally, given the large number of outcomes assessed, the possibility of these results occurring by chance was evaluated. For the behavioral measures (shown in Tables 3a and 3b), it is noteworthy that statistically significant associations with urinary Hg that were in the expected direction were observed for 15/20 tests among DD and 11/20 tests among DA. The pattern of associations with urinary Hg was also consistent with our established a priori hypothesis that performance scores within the domains of attention, Hg, and motor function are potentially associated with exposure to Hg° (Clarkson, 2002). For example, within the domains for memory, all four measures were affected by exposure to Hg among DD and two out of four among DA. Within the domain for motor function, all four measures were adversely affected among DD and DA. Furthermore, no statistically significant associations between Hg and behavior were found to be in the unexpected direction.

DISCUSSION

The basis for selecting the domains evaluated in this study was dependent on evidence of declines in neurobehavioral performance test scores among clinical cases of Hg° toxicity coupled with experimental and occupational evidence of impairment from Hg° exposure. It was anticipated that environmentally induced declines would be consistent with a significant linear exposure–effect response. Classic signs of mercurialism (Vroom & Greer, 1972) include

(1) psychosomatic symptoms (salivation, insomnia, and loss of appetite); (2) alterations in affect or emotional lability (mood swings, irritability, fatigue, loss of interest, withdrawal, and sweating and blushing, known as erethism); (3) insidious loss of mental capacity (progressively affecting memory, logical reasoning, or intelligence); and (4) motor effects (in the arms, progressing to incoordination, imbalance, and cerebella ataxia and tremor in muscles that are highly enervated and perform fine motor control of extremities, such as fingers, eyelids, and lips) (Gerstner & Huff, 1977a). The diversity in observed nervous-system effects indicates more than one mechanism of toxicity and involvement of more than one area of the brain. For example, exposure to Hg° may interfere with the limbic system associated with mood and memory, the motor strip associated with movement, and peripherally insult axons associated with vibration sensitivity or visual perception. In keeping with these diverse effects, our Hg-related hypothesis was that exposure to low levels of Hg may (1) increase mood scales, (2) deteriorate cognitive skills requiring prolonged attention, memory, and psychomotor skills, and (3) reduce motor speed. It was postulated that these are selective effects, leaving language and retrograde memory intact (White et al., 1992). The results for symptoms in relation to the 5-HTTLPR polymorphism and Hg exposure were previously described (Heyer et al., 2009).

In this study the remaining three domains were reevaluated in light of the potential effects of the genetic variant 5-HTTLPR. Our 5-HTTLPR-related hypothesis was that it would be highly associated with mood consistent with its pathogenic role in anxiety, agitation, and depression (Yoshida et al., 2002). The new question addressed in this study is whether the effect of the genetic variant operates more broadly to moderate performance scores in other behavioral domains known to be affected by exposure to Hg.

In this study population, the distribution of the 5-HTTLPR polymorphism was 31% wild type (I/I, without deletion on either allelle), 50% heterozygous (l/s, no deletion on allelle 1 and deletion on allelle 2), and 19% mutant (s/s, deletion on both allelles), which is comparable to that observed in other healthy human populations (Lesch et al., 1996). The gender distribution for the l/s + s/s allellic combination was more prominent among females than males, 60% in DD and 80% in DA (Table 1), which is not commonly reported in the literature and therefore reflects the composition of this population.

Exposure to Mercury and the Presence of the 5-HTTLPR Polymorphism Independently Alter Behavioral Performance

A central question addressed here is whether subgroups of individuals with the 5-HTTLPR polymorphism may be at greater risk for Hg-mediated CNS effects at comparable exposure levels. In DD and DA the most convincing increase in declines attributable to the genetic variant in the presence of Hg was for scores within the domain of manual coordination comprised of scores from the BEES Finger Tapping $\text{Test}_{\text{Dom//Non-dominant and Alternate}}$, the Hand Steadiness $\text{Battery}_{\text{Factor1}}$, and the BEES Manual Tracking Test, suggesting a potential disruption in meeting the demands of manual coordination tasks discussed in more detail later in this article. In DD, only one other test score for cognitive flexibility (the BEES $\text{Switching}_{\text{Rate}}$) was also jointly affected. In DA, statistically significant coexposures to Hg° and the genetic variant also included measures for cognitive flexibility and manual coordination. However, joint effects were broader, encompassing other test scores in three different domains (attention, sustained attention, and perception). They include the BEES Digit $\text{Span}_{\text{Forward N Digits}}$, the BEES $\text{Vigilance}_{\text{Hits}}$, and the BEES Pattern $\text{Discrimination}_{\text{Latency (secs)}}$.

It is striking that manual dexterity scores were adversely affected in both genders for the $\text{BEES}_{\text{Finger Tapping N Taps}}$ and Hand $\text{Steadiness}_{\text{Factor1}}$. In line with Kahneman's capacity model (Kahneman, 1973), one psychophysical explanation partially explaining this observation is that performance on the finger speed, hand steadiness, and manual tracking is physically more demanding. It takes more effort or attention to complete the task, which heightens arousal and

tension, than on performance on other cognitive tests. It was postulated that the increase in physical or mental demand causes reallocation of attentional resources subject to individual differences in degradation of performance. It is noteworthy that exposure to Hg° also alters short-term attention as measured by Digit $\text{Span}_{\text{Forward N Digits}}$ in this study. A similar conclusion was reported in a behavioral study in mice (Ansorge et al., 2004). In that study, reduced locomotor activity seen in an open field and an elevated plus-maze condition were similarly interpreted to be more likely related to the novel stress of the environment rather than frank motor dysfunction associated with the 5-HTTLPR polymorphism, particularly since no differences in locomotion ability were seen when mice were assessed in their home cage. Alternatively, adverse motor coordination associations may reflect observed declines in other dependent domains within attention, sustained attention, or cognitive flexibility that were also affected by exposure to the polymorphism.

The Presence of the 5-HTTLPR Polymorphism Independently Alters Other Behavioral Performance

Among DD, one additional statistically significant decline in performance score was observed with 5-HTTLPR in the expected direction for the BEES Pattern $\text{Discrimination}_{\text{N Correct}}$ but not its latency. In contrast, among DA several more statistically significant declines were observed across test scores in working memory (the BEES Digit and Spatial $\text{Span}_{\text{Backward N Digits}}$), visual memory (the BEES Pattern Memory $_{\text{Latency (msecs)}}$), cognitive flexibility (the BEES $\text{Switching}_{\text{Mean Latency (msecs)}}$), and reaction time (the BEES Simple and Choice Reaction $\text{Time}_{\text{Lift (msecs)}}$). It is noteworthy that scores for the BEES Simple and Choice Reaction $\text{Time}_{\text{Move (msecs)}}$ remained intact, suggesting that the simple movement response in Simple or Choice Reaction $\text{Time}_{\text{Move (secs)}}$ ($R = -0.06$ or -0.09) is not as cognitively demanding as lifting one's finger in response to stimuli in the Simple and Choice Reaction $\text{Time}_{\text{Lift (secs)}}$ ($R = -0.18$ or -0.28). A similar observation was found with the increasing strength of associations between the variant and sets of $\text{Switching}_{\text{Latency (secs)}}$ scores. The standardized beta in regression models increases with greater cognitive demand, which is likely associated with the novelty or complexity of the task. The BEES Switching task is an application of the Posner Switching paradigm (Posner & Petersen, 1990), where it is thought that the decision to agree between two familiar letters ($R = -0.15$) is easier than the demand of deciding whether 2 unfamiliar patterns agree $R = -0.22$) or disagree. Both of these comparison tasks, in turn, are thought to be less demanding than determining whether the position of a black dot displayed on the screen either agrees or disagrees with the displayed word below it for a "left" or "right" side of the square ($R = -0.25$).

The absence of consistency of 5-HTTLPR-related effects between genders is subject to interpretation. One possible explanation partially accounting for the limited behavioral expression of the polymorphism among DD, in contrast to DA, is that dentists have greater performance reserve attributable to more uniform and higher professional education and occupational training, which could mask the effect of the polymorphism in test performance (see Table 1). This explanation also suggests that the behavioral expression of the polymorphism is sensitive to individual differences between the two groups. Collectively, these findings, though limited in DD, still identify 5-HTTLPR as a genetic factor that independently affects performance on measures that are also modified by exposure to Hg, resulting in greater vulnerability from exposure to Hg alone.

Exposure to Mercury and the Presence of the 5HTTLPR Polymorphism Independently Alter Mood Scores

Affective symptomology was measured by the Profile on Mood States, the Beck Depression Index, and the SCL-90. The joint contribution of exposure to Hg° and the genetic variant on mood scores among male DD was sparse. Out of a maximum of 14 measures, only the subscore

for POMS Confusion, the subscore SCL-$90_{Factor\ 2}$ for Listlessness, and the sum score for BDI were independently affected by both exposures. In contrast, among female DA, 7 out of 14 subscores were jointly affected by both exposures in the expected direction: 4 of 6 measures for the POMS, 2 of 4 factors for the BDI, and 2 of 4 factors for the SCL-90, but one other effect of the variant on SCL-$90_{Factor4}$ for guilt was in the unexpected direction. Of equal importance, in both DD and DA, the strength of 5-HTTLPR-related associations was equivalent across subscores within and across instruments, suggesting the underlying response to the genetic variant is non-specific and sensitive to individual differences.

Consistent with the pathogenic role of 5-HTTLPR in anxiety, agitation, and depression (Yoshida et al., 2002), these findings confirm that the variant is strongly associated with mood scores in adults, but its presence does not alter Hg-related susceptibility. Current studies are in progress to reevaluate similar associations among children exposed to Hg amalgam.

Newly Observed Hg-Related Exposure-Effect Relationships

In general, when adding the term for 5-HTTLPR polymorphic status to analytic models, previous reporting of Hg°-related neurobehavioral declines in performance was replicated (Echeverria et al., 2005, 2006; Heyer et al., 2004) at urinary Hg concentrations below 4 μ/L. Statistically significant decreases in performance were demonstrated across domains considered vulnerable to Hg° insult (Anger, 1985; Echeverria et al., 1995) in the absence of reduced general intelligence as measured by the BEES Vocabulary and the TONI-3 (Brown et al., 1997).

However, notable additions were also observed. In DD, the addition of the variant in models resulted in newly reported statistically significant associations with Hg for one test for sustained attention (BEES $Vigilance_{Hits}$) and perception (BEES Pattern $Discrimination_{Latency\ (msecs)}$), one of two tests for higher order cognitive flexibility functions (the BEES $Switching_{Latency\ (msecs)}$, but not the BEES Trail-making $B_{Latency\ (secs)}$, as well as that for the BEES Simple and Choice Reaction $Time_{Move\ (secs)}$.

In these cases, 5-HTTLPR status was only slightly correlated with behavior and improved the model "*R* values" directly by predicting some of the variance in the dependent variable and indirectly by clarifying the associations with Hg. The pattern suggests that the polymorphism likely acts like suppressor variables (Cohen & Cohen, 1975), where control for the emotional demand required to complete these tests allowed urinary Hg to explain more of the variance in the behavioral measure itself. This is because suppressor variables typically remove variance in the outcome due to measurement artifacts. Otherwise, most Hg-related declines in performance scores assigned to the domains of attention, working memory, visual memory, and manual coordination (Echeverria et al., 2005, 2006; Heyer et al., 2004) were replicated.

CONCLUSIONS

These results confirm that the variant for 5-HTTLPR is highly associated with particpants' mood states and that the polymorphism operates independently of Hg to moderate performance scores in behavioral domains known to be affected by exposure to Hg. Further, controlling for 5-HTTLPR status did unmask previously unaffected performance scores within domains for sustained attention and perception in both genders, and other performance scores in cognitive flexibility and reaction time in DD alone. These findings add to existing evidence of genetic determinants of mood and behavior that potentially increase susceptibility to Hg toxicity in human subjects (Echeverria et al., 2005, 2006; Heyer et al., 2004, 2008, 2009). Ongoing studies are directed toward replication and further characterization of these associations in children with low-level Hg° exposure.

Acknowledgments

This work was supported in part by grant R01DE11712 from the National Institute of Dental and Craniofacial Research (NIDCR), by the University of Washington Center for Ecogenetics and Environmental Health grant P30ES07033, and by the University of Washington Superfund Program Project grant P42ES04696 from the National Institute of Environmental Health Sciences (NIEHS). Additional support was provided by the Wallace Research Foundation.

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TABLE 1

Study Population Traits, Percentage and/or Mean (SD)

Trait	Dentists (*n* = 164)	Dental assistants (*n* = 101)
Caucasian	96%	83%
Age at evaluation	48.8 (7.7)	36.0 (8.8)
Income in $1000s	166.4 (103)	45.9 (26.0)
BEES Vocabulary, N Corr. (*n* = 12)	10.6 (0.9)	8.2 (1.9)
Reading Test for the Word Pronunciation Test (WRAT3)	52.6 (3.1)	42.0 (3.0)
Test of Nonverbal lntelligence-3 (TONI-3)	34.4 (6.6)	28.6 (8.6)
Highest Academic Education Score	7.0 (1.1)	4.7 (0.9)
Snell Equivalent Right	1.55 (1.8)	1.78 (1.96)
Number of alcohol drinks per week	4.1 (4.7)	1.7 (2.3)
Number of fish meals per week	1.7 (0.7)	1.2 (0.7)
Medical history: % (n)		
Use prescription drugs	29% (n=48)	59% (n=60)
Have a current physical impairment	6% (n=10)	10% (n=10)
Kidney problems	5% (n=7)	5% (n=5)
Antidepressant medications	5% (n=8)	11.9% (n=12)
Exposure: mean (SD)		
Urinary mercury (HgU in µg/L)	2.52 (2.22)	1.98 (1.98)
Years exposed to mercury in dentistry	19.10 (10.37)	10.03 (7.64)
Number of mercury amalgam restorations	16.03 (15.66)	12.34 (10.77)
Chronic Index $_{(Yrs*Type*\# restorations/wk *Decade)}$	1212 (1877)	316 (429)
5-HTTLPR genotype distribution		
Homozygous common (I/I)	40%	20%
Heterozygous (l/s)	40%	56%
Homozygous (s/s)	20%	24%

TABLE 2

Mean (SD) for Behavioral Test Domains

Behavioral domains[a]	Dentists (*n* = 164), mean (SD)	Dental assistants (*n* = 101), mean (SD)
Attention (*n* = 3 tests)		
Digit Span$_{\text{Forward N Digits}}$	5.5 (1.3)	5.0 (1.1)
Spatial Span$_{\text{Forward N Digits}}$	5.4 (1.07)	5.1 (1.0)
Trailmaking A$_{\text{Latency (secs)}}$	21.0 (5.6)	19.0 (4.6)
Visual Memory (*n* = 2 tests)		
Visual Reproduction$_{\text{N Correct}}$	36.22 (3.44)	32.65 (5.34)
Pattern Memory$_{\text{N Correct}}$	18.9 (1.1)	18.2 (1.1)
Pattern Memory$_{\text{Latency (sec)}}$	4.46 (1.52)	4.63 (1.62)
Working Memory (*n* = 2 tests)		
Digit Span$_{\text{Backward N Digits}}$	3.88 (1.4)	3.34 (1.13)
Spatial Span$_{\text{Backward N Digits}}$	4.98 (1.1)	4.48 (0.95)
Visuomotor Processing (*n* = 1 test)		
Symbol Digit$_{\text{Latency for 9 substitutions (secs)}}$	2.92 (0.55)	2.88 (0.54)
Sustained Attention (*n* = 1 test)		
Vigilance$_{\text{Hits (max=25)}}$	43.5 (1.5)	43.5 (1.3)
Perception (*n* = 1 test)		
Pattern Discrimination$_{\text{Latency (secs)}}$	6.54 (1.9)	5.98 (2.0)
Cognitive Flexibility (*n* = 2 tests)		
Switching$_{\text{Mean Latency for Letter/Pattern/Direction (secs)}}$	2.21 (0.51)	2.08 (0.45)
Trailmaking B$_{\text{Latency (secs)}}$	42.0 (12.7)	44.1 (14.0)
Reaction Time (ms) (*n* = 2 tests)		
S. Reaction Time$_{\text{Lift msecs}}$	302.6 (40.1)	287.7 (35.6)
Choice Reaction$_{\text{Lift msecs}}$	421.3 (42.6)	406.6 (43.3)
S. Reaction Time$_{\text{Move msecs}}$	467.9 (144.2)	552.9 (198.5)
Choice Reaction$_{\text{Move msecs}}$	709.4 (192.6)	864.5 (270.7)
Manual Dexterity Skills (*n* = 3 tests)		
Finger Tap$_{\text{Dom//Non-dom Taps/10secs}}$	61.3 (9.1)	55.7 (10.5)
Finger Tap$_{\text{Alternate Partialed Taps/10 sec}}$	56.5 (23.0)	43.2 (18.6)
Hand Steadiness$_{\text{Factor 1}}$	0.27 (0.91)	0.62 (1.07)
Tracking Score$_{\text{Median Frequency}}$	0.77 (0.11)	0.62 (0.15)
Sensory (*n* = 1 test)		
Vibration Sensitivity$_{\text{TLV Score}}$	14.3 (8.4)	10.3 (2.2)
Affect Symptomology		
Profile on Mood States (*n* = 6)		
POMS Tension Scale	5.31 (4.32)	8.23 (6.05)
POMS Depression Scale	3.27 (5.59)	6.19 (7.59)
POMS Anger Scale	3.87 (5.25)	6.05 (5.94)

Behavioral domains[a]	Dentists (*n* = 164), mean (SD)	Dental assistants (*n* = 101), mean (SD)
POMS Vigor Scale	19.29 (5.01)	14.65 (5.91)
POMS Fatigue Scale	5.22 (4.06)	7.77 (5.39)
POMS Confusion Scale	3.11 (2.79)	4.78 (3.19)
POMS Overall Total Scale	40.07 (17.23)	47.67 (21.25)
Beck Depression Index Sum (*n* = 4)	3.27 (3.78)	7.86 (6.73)
BDI Factor 1 Worthlessness	−0.20 (0.80)	0.29 (1.18)
BDI Factor 2 Listless	−0.14 (0.79)	0.55 (1.39)
BDI Factor 3 Anxiety	−0.02 (0.73)	0.10 (1.05)
BDI Factor 4 Depression	−0.25 (0.74)	0.19 (1.62)
Symptom Checklist 90 (SCL-90) Sum (*n* = 4)	18.94 (20.30)	42.78 (35.48)
SCL90 Factor 1 Distrustful	−0.01 (1.03)	0.11 (1.00)
SCL90 Factor 2 Listless	0.09 (1.42)	0.15 (1.04)
SCL90 Factor 3 Fearful	0.15 (1.11)	0.01 (1.08)
SCL90 Factor 4 Guilty	0.00 (0.83)	0.03 (0.76)

[a] *n*, Number of tests used to evaluate each behavioral domain.

TABLE 3

TABLE 3a. Multiple Regression Model Coefficients[a] Demonstrating Simultaneous Associations Between Behavioral Domains,[b] Urinary Mercury, and the 5-HTTLPR Variant

	Urinary Hg		5-HTTLPR$_{Variant}$			
Dentists (n = 164)	**Beta**	***p***	**Beta**	***p***	**Magnitude of additive effect**	**Fold Change**
Attention (n = 3 tests)						
Digit Span$_{Forward\ N\ Digits}$	−0.21	*.002*		ns		
Visual memory (n = 2 tests)						
Visual Reproduction $_{N\ Correct}$	−0.20	*.008*		ns		
Pattern Memory$^{c}{}_{Latency\ (sec)}$	−0.20	*.008*		ns		
Working memory (n = 2 tests)						
Digit Span$_{Backward\ N\ Digits}$	−0.16	*.03*		ns		
Spatial Span$_{Backward\ N\ Digits}$	−0.15	*.05*				
Visuomotor Processing (n = 1 test)						
Symbol Digit$^{c}{}_{Latency\ for\ 9\ substitutions\ (secs)}$	−0.19	*.02*		ns		
Sustained attention (n = 1 test)						
Vigilance$^{c}{}_{Hits\ (max\ =\ 25)}$	−0.10	*.04*		ns		
Perception (n = 1 test)						
Pattern Discrimination$^{c}{}_{N\ Correct}$		ns	−0.15	*.01*		
Pattern Discrimination$^{c}{}_{Latency\ (secs)}$	−0.22	*.008*		ns		
Cognitive flexibility (n = 2 tests)						
Switching$^{c}{}_{Mean\ Latency\ for\ Pattern\ (secs)}$	−0.14	*.006*	−0.12	*.04*	−0.033	0.04
Reaction time (n = 2 tests)						
S. Reaction Time$^{c}{}_{Move\ msecs}$	−0.18	*.007*		ns		
Choice Reaction Time$^{c}{}_{Move\ msecs}$	−0.21	*.002*		ns		
Manual coordination skills (n = 3 tests)						
Finger Tap$^{c}{}_{Dom/Non\text{-}dominant\ Taps/10\ sec}$	−0.17	*.02*	−0.14	*.04*	−0.08	0.13
Finger Tap$^{c}{}_{Alternate\ Partial\ Taps/10\ sec}$	−0.15	*.03*	−0.18	*.03*	−0.10	0.14
Hand Steadiness$_{Factot1}$	−0.16	*.04*	−0.19	*.01*	−0.60	0.10

TABLE 3a. Multiple Regression Model Coefficients[a] Demonstrating Simultaneous Associations Between Behavioral Domains,[b] Urinary Mercury, and the 5-HTTLPR Variant

	Urinary Hg		5-HTTLPR$_{Variant}$			
Dentists (n = 164)	**Beta**	***p***	**Beta**	***p***	**Magnitude of additive effect**	**Fold Change**
Tracking Score[c]$_{Median\ Frequency}$	−0.14	*.04*		ns		
Sensory (n = 1 tests)						
Vibration $_{TLV\ Score}$ (with repetitive trauma)	−0.11	*0.04*		ns		
Vibration $_{TLV\ Score}$ (exclude repetitive trauma)		ns		ns		

TABLE 3b. Multiple Regression Model Coefficients[a] Demonstrating Simultaneous Associations Between Behavioral Domains,[b] Urinary Mercury, and the 5-HTTLPR Variant

	Urinary Hg		5-HTTLPR$_{Variant}$		Magnitude of additive effect	
Dental assistants (n = 101):	**Beta**	***p***	**Beta**	***p***		**Fold change**
Attention (n = 3 tests)						
Digit Span$_{Forward\ N\ Digits}$	−0.13	*.01*	−0.18	*.04*	*−0.60*	*0.11*
Working memorry (n = 2 test)						
Digit Span$_{Backward\ N\ Digits}$	−0.13	*.01*	−0.20	*.03*	*−0.61*	*0.15*
Spatial Span$_{Backward\ N\ Digits}$		ns	−0.24	*.01*		
Visual memory (n = 2 tests)						
Visual Reproduction$_{N\ Correct}$	−0.16	*.03*		ns		
Pattern Memory$^{+}_{Latency\ (sec)}$		ns	−0.24	*.006*		
Visumotor processing (n = 1 test)						
Symbol Digit$^{+}_{Latency\ for\ 9\ substitutions\ (secs)}$	−0.20	*.02*				
Sustained attention (n = 1 test)						
Vigilance$_{Hits\ (max\ =\ 25)}$	−0.22	*.04*	−0.31	*.008*	*−0.05*	*0.01*
Perception (n = 1 test)						
Pattern Discrimination$_{N\ Correct}$	−0.16	*.04*		ns		
Pattern Discrimination$_{Latency\ (secs)}$	−0.11	*.02*	−0.26	*.03*	*−0.02*	*0.55*
Cognitive flexibility (n = 2 tests)						
Switching$^{+}_{Mean\ Latency\ for\ Letter/Pattern/Direction\ (secs)}$		ns	−0.26	*.004*		
Switching$^{+}_{Mean\ Latency\ for\ Letter\ (secs)}$		ns	−0.15	ns		

TABLE 3b. Multiple Regression Model Coefficients[a] Demonstrating Simultaneous Associations Between Behavioral Domains,[b] Urinary Mercury, and the 5-HTTLPR Variant

	Urinary Hg		5-HTTLPR$_{Variant}$		Magnitude of additive effect	
Dental assistants (n = 101):	**Beta**	***p***	**Beta**	***p***		**Fold change**
Switching$^{+}_{\text{Mean Latency for Picture (secs)}}$		ns	−0.22	*.02*		
Switching$^{+}_{\text{Mean Latency for Direction (secs)}}$		ns	−0.25	*.005*		
Trailmaking B$^{+}_{\text{Latency (secs)}}$	−0.16	*.01*		ns		
Reaction time (n = 2 tests)						
S. Reaction Time$^{+}_{\text{Lift msecs}}$		ns	−0.28	*.003*		
Choice Reaction$^{+}_{\text{Lift msecs}}$		ns	−0.18	*.04*		
S. Reaction Time$^{+}_{\text{Move msecs}}$		ns	−0.06	ns		
Choice Reaction$^{+}_{\text{Move msecs}}$		ns	−0.09	ns		
Manual coordination skills (n = 3 tests)						
Finger Tap$^{+}_{\text{Dom//Non-dominant Taps/10 sec}}$	−0.13	*.04*		ns		
Finger Tap$^{+}_{\text{Alternate Partial Taps/10 sec}}$	−0.13	*.05*	−0.20	*.02*	−0.35	4.76
Hand Steadiness$_{\text{Factor1}}$	−0.19	*.02*	−0.18	*.03*	−0.47	199.0
Tracking Score$^{+}_{\text{Median Frequency}}$	−0.20	*.05*	−0.25	*.02*	−0.087	0.15
Sensory (n = 1)						
Vibration Sensitivity$_{\text{TLV Score}}$	−0.17	*.03*		ns		

[a] Regression models for dentists assessed urinary mercury, the 5HTTLPR polymorphism, and their interaction, controlling for age, number of alcoholic drinks per week, and premorbid intelligence (vocabulary).

[b] n, Number of tests used to evaluate each behavioral domain; ns, not significant, $p < .05$.

[c] BEES Scores were transformed in a standardized manner (Echeverria et al., 2002, 2005, 2006).

[a] Regression models for dental assistants assessed urinary mercury, the 5HTTLPR polymorphism, and their interaction, controlling for age, number of alcoholic drinks per week, premorbid intelligence (vocabulary), and level of education.

[b] n, Number of tests used to evaluate each behavioral domain; ns, not significant, $p < .05$.

[c] BESS Scores were transformed in a standardized manner (Echeverria et al., 2002, 2005, 2006).

TABLE 4

Multiple Regression Model Coefficients[a] Demonstrating Simultaneous Associations Between Affect (Profile on Mood States, BDI, and SCL-90), Urinary Mercury, and 5-HTTLPR Variant

Dentists (*n* = 164)						
	Urinary Hg		5-HTTLPR$_{Variant}$		Magnitude of additive effect (difference)	Fold change
Test and affect	**Beta**	***p***	**Beta**	***p***		
Profile on Mood States (*n* = 6)						
Anger		ns	0.24	*.000*		
Depression		ns	0.22	*.001*		
Tension		ns	0.16	*.02*		
Confusion	0.13	*.02*	0.19	*.01*	0.92	0.22
Vigor		ns	0.12	*.04*		
Total		ns	0.21	*.001*		
Beck Depression Index (*n* = 4)						
BDI Sum	0.29	*.02*		ns		
BDI Factor 1 Listless		ns	0.26	*.001*		
BDI Factor 2 Worthlessness		ns	0.24	*.007*		
BDI Factor 3 Depression		ns	0.23	*.005*		
BDI Factor 4 Agitation		ns	0.23	*.006*		
The Symptom Checklist-90 (*n* = 4)						
SCL90 Sum	0.18	*.03*		ns		
SCL90 Factor 2 Listless	0.16	*.05*	0.19	*.04*	0.66	1.27
SCL90 Factor 3 Fearful		ns	0.17	*.04*		
Dental assistants (*n* = 101)						
Profile on Mood States (*n* = 6)						
Anger	0.19	*.03*	0.19	*.05*	1.39	0.36
Depression			0.23	*.02*		
Tension	0.19	*.04*	0.18	*.05*	2.67	0.25
Confusion		ns	0.28	*.02*		
Fatigue	0.20	*.05*	0.32	*.005*	3.82	0.39

Dentists (n = 164)						
	Urinary Hg		5-HTTLPR$_{Variant}$		Magnitude of additive effect (difference)	Fold change
Test and affect	**Beta**	***p***	**Beta**	***p***		
Vigor	−0.21	*.03*	−0.25	*.01*	−1.98	0.14
Total		ns	0.23	*.02*		
Beck Depression Index (n=4)						
BDI Sum			0.35	*.01*		
BDI Factor 1 Listless	0.34	*.003*	0.55	*.000*	0.168	0.11
BDI Factor 2 Worthlessness	0.15	*.03*	0.18	*.04*	0.168	0.13
The Symptom Checklist-90						
SCL90 Sum		ns	0.25	*.04*		
SCL90 Factor 1 Distrustful		ns	0.28	*.01*		
SCL90 Factor 3 Fearful	0.33	*.02*	0.45	*.009*	1.27	1.49
SCL90 Factor 4 Guilty	0.31	*.03*	(−0.25)	*.04*	(−0.77)	(2.37)

[a]Regression models are restricted to Race = white. For dentists, models control for age, number of alcoholic drinks per week, and premorbid intelligence (vocabulary). Regression models for dental assistants also include the level of education; analyses in the unexpected direction are noted by parentheses.