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Evaluating the Role of Serotonin on Neuropsychological Function After Breast Cancer Using Acute Tryptophan Depletion

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Abstract

Although cognitive dysfunction is a prevalent and disruptive problem for many breast cancer survivors (BCSs), little research has examined its etiology. One potential mechanism that remains to be explored is serotonin. Serotonin has been implicated in normal and dysfunctional cognitive processes, and serotonin levels are significantly affected by estrogen withdrawal, a common side effect of breast cancer treatment. However, no study has evaluated serotonin's role on cognitive dysfunction in BCSs. The purpose of this study was to examine the role of serotonin in cognitive dysfunction in survivors by lowering central serotonin concentrations via acute tryptophan depletion (ATD). Based on previous research in noncancer populations, we hypothesized that alterations in central serotonin levels would induce cognitive dysfunction in these women controlling for confounding characteristics such as fluctuating mood and glucose levels. Secondly, we explored whether genetic variations in serotonin genes would partly explain ATD. Participants included 20 female BCSs, posttreatment for nonmetastatic breast cancer, who received ATD or control in a double-blind, crossover design. Cognitive performance was measured at the 5-hr tryptophan/serotonin nadir on each test day using standardized neuropsychological tests. Specific impairment was noted in episodic memory (delayed recall) and motor speed during ATD versus control. ATD did not alter new learning (immediate recall), working memory, verbal fluency, or information processing speed. Findings suggest that serotonin may play a critical role in memory consolidation and motor functioning in BCSs.

Keywords

attention; breast cancer; cognition; serotonin; tryptophan depletion

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Declaration of Conflicting Interests

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Breast cancer survivors (BCSs) comprise the largest group of cancer survivors, with over 2.4 million female survivors estimated to be living in the United States alone (Altekruse et al., 2010). Up to 83% of BCSs report some degree of cognitive impairment (Jenkins et al., 2006), and this impairment has been shown to be significantly correlated with depressive symptoms (Von Ah, Russell, Storniolo, & Carpenter, 2009). Clinically significant cognitive impairments have also been noted on objective neuropsychological tests (Von Ah, Harvison, et al., 2009). Although cognitive deficits may be disruptive, bothersome, and potentially debilitating, few studies have explored their etiology.

One potential mechanism that remains to be explored is serotonin. Serotonin is a monoamine neurotransmitter that is synthesized, stored, and released by specific neurons in the brain. Within the brain, the serotonin pathway originates from neurons in the dorsal and ventral raphe nuclei and projects to the majority of the forebrain areas involved with cognitive functioning. Serotonergic pathways and receptors have been implicated in brain areas involved with normal and dysfunctional cognitive processes (Meneses, 1999; Schmitt, Wingen, Ramaekers, Evers, & Riedel, 2006). For example, Alzheimer's disease has been associated with decrements in serotonin markers such as the uptake/transporter complex and a number of serotonin receptors (Meneses, 1999), and low serotonin activity has been linked to the cognitive deficits commonly seen in both Alzheimer's disease (Meneses, 1999; Porter, Lunn, & O'Brien, 2003) and depression (Riedel, 2004).

BCSs may be at increased risk of cognitive impairment. Research suggests that serotonin is significantly affected by estrogen levels (Halbreich et al., 1995), and estrogen withdrawal is a common side effect of breast cancer treatments such as chemotherapy, selective estrogen receptor modulators, and aromatase inhibitors (Ahles et al., 2002). Estrogen appears to directly affect serotonin neurons regulating genes involved in serotonin synthesis, transport, and signaling (Mohyi, Tabassi, & Simon, 1997). Low serotonin levels have been observed in women after spontaneous or surgical menopause, and estrogen replacement has been shown to restore serotonin concentrations (Blum et al., 1996). However, the role of serotonin in the cognitive functioning of BCSs has yet to be explored.

One method used recently to evaluate the role of central serotonin transmission in cognitive functioning is the acute tryptophan depletion (ATD) paradigm (Mendelsohn, Riedel, & Sambeth, 2009). The ATD paradigm has been used widely within the field of psychiatry, primarily to examine the role of central serotonin transmission in mood disorders (Ruhé, Mason, & Schene, 2007). ATD temporarily reduces central tryptophan and serotonin by (1) inducing anabolism of essential amino acids by the liver and, thus, depleting circulating serum tryptophan and (2) altering the ratio of large neutral amino acids (LNAA) for blood-brain transport, resulting in lower levels of tryptophan (a precursor of serotonin) and, ultimately, a reduction in serotonin metabolism (Booij, Van der Does, & Riedel, 2003). ATD can be used safely without serious medical or psychological complications (Booij et al., 2003), its effects on central serotonin in humans have been confirmed through cerebral spinal fluid sampling (Salomon et al., 2003), and its effects are specific for serotonin, with no direct effects on other neurotransmitters (Booij et al., 2003). ATD is accomplished by administering a 100 g amino acid drink that contains no tryptophan. Central tryptophan becomes temporarily depleted as protein synthesis uses up the existing tryptophan, resulting in temporary lowering of central serotonin neurotransmission within 4.5 to 7 hr (Booij et al., 2003).

While the impact of serotonin on mood and emotion has been well established, its role in cognitive dysfunction is not thoroughly understood. Serotonin has been implicated as playing a role in a number of cognitive processes, most commonly learning and memory. However, research results have been mixed. Seven small studies have been conducted

examining declarative episodic verbal memory using the Rey Auditory Verbal Learning Test (RAVLT; Mendelsohn et al., 2009). Of these, two found significant impairment with delayed recall (Merens, Booij, Haffmans, & Van der Does, 2008; Porter et al., 2005) and three found some impairment in immediate recall (Hayward, Goodwich, Cowen, & Harmer, 2005; Merens et al., 2008; Porter et al., 2005), while the remaining four studies did not find significant impairment in either immediate or delayed recall (Hughes et al., 2003; Kulz, Meinzer, Kopasz, & Voderholzer, 2007; Porter et al., 2003; Shansis et al., 2000). Interestingly, significant findings were predominately noted in samples that may be classified as having a “serotonergic vulnerability” (pooled sample of healthy elderly, recovered depressed patients, and remitted depressed patients; Mendelsohn et al., 2009). Collectively, these findings suggest that serotonin may be involved with verbal learning tasks (immediate and delayed recall), especially in vulnerable populations. In addition, while some ATD studies have documented decrements in working memory, verbal fluency, information processing speed, and psychomotor ability, others have not. As a result of these inconsistent findings, Mendelsohn, Riedel, and Sambeth (2009) have called for continued research to investigate the effects of ATD on multiple cognitive domains to further elucidate the role of the serotonergic system on cognition, especially in potentially vulnerable populations. Additional studies must build on and address limitations noted in previous ATD studies, including examining the impact of known confounding variables on cognition such as mood and blood glucose levels. While most ATD studies have collected and reported mood levels (80%), none have reported fluctuations in blood glucose levels.

Another important consideration is the role of individual variability in ATD. The variability in response to ATD could be partly explained by inherited polymorphisms in serotonin receptor and synthesis genes (Lane et al., 2008; Moreno et al., 2001, 2002). In fact, Porter and colleagues (2005), who studied 33 elderly and recovered depressed patients using ATD, identified failure to genotype participants as a limitation in understanding study results. In the study described here, we explored whether the variability of three candidate genes involved in serotonin signaling and encoding serotonin receptors (*HTR1A* and *HTR2A*) and tryptophan hydroxylases (*TPH1*) would partly explain variability in ATD. The *HTR1A* and *HTR2A* receptors are involved in the neurotransmission of serotonin effects and tryptophan hydroxylases catalyzes the rate-limiting step in the biosynthesis of serotonin. Allelic variations in all three genes have been associated with various antidepressant responses (Murphy, Kremer, Rodrigues, & Schatzberg, 2003). In summary, specific polymorphisms in serotonin receptor and synthesis genes may alter ATD response.

Therefore, the primary purpose of this study was to evaluate the role of serotonin on cognitive functioning using ATD in BCSs, a group of women who may be at increased risk of cognitive dysfunction due to rapid estrogen withdrawal as a result of breast cancer treatment. Based on research in noncancer populations, we hypothesized that deficits in central serotonin would induce cognitive deficits, including decreases in immediate and delayed recall, working memory, verbal fluency, speed of processing, and psychomotor ability. Secondly, to build on and address limitations of previous research, we evaluated characteristics that may influence cognitive functioning, including mood and blood glucose levels, and explored the influence of genetic polymorphisms in three candidate genes on responses to ATD in BCSs. Based on previous research, we hypothesized that inherited polymorphisms in *HTR1A*, *HTR2A*, and *TPH1* genes could partly explain variability in response to ATD.

Materials and Method

Participants

We recruited 20 female BCSs from an outpatient cancer clinic. Participants were aged 30 to 66 years ($M = 51.2$ years, $SD = 9.3$ years), were postmenopausal (> 12 months amenorrhea), had been successfully treated for nonmetastatic breast cancer (i.e., Stage IV or less), and were considered to be free of cancer at the time of the study. We assessed physical and mental health of each subject by means of a health questionnaire, laboratory analyses, and structured clinical assessment with a psychologist. Women were excluded who had a history of or current depression (24-item Hamilton Depression Rating Scale, HAM-D score ≥ 18 ; Hamilton, 1960), a history of migraines, a history of hepatitis, or an abnormal chemistry profile (i.e., sodium, potassium, glucose). We did not exclude women taking antiestrogen, selective serotonin reuptake inhibitors (SSRIs), or selective norepinephrine reuptake inhibitors (SSNRIs) because (1) previous research has shown that usage of SSRIs/SSNRIs did not affect adequate ATD in menopausal women (Epperson et al., 2007), (2) a large percentage of the BCS population takes antiestrogens or SSRIs/SSNRIs. SSRIs/SSNRIs are often taken for other symptoms (e.g., hot flashes), and (3) the study design (within subject) controls for their usage. The Cancer Center Scientific Review Committee and the University Institutional Review Board approved the study. All participants gave their written informed consent prior to participation.

Study Design

The study used a within-subject, double-blind, placebo-controlled balanced crossover design. The treatment consisted of administration of a 100 g placebo or ATD mixture. Treatment order was randomly assigned and balanced over the 2 test days, which were 7 days apart.

Composition of Amino Acid Suspension

ATD was achieved with a 300 mL amino acid drink and encapsulated amino acids containing 100 g total amino acids in the following ratio: L-alanine 5.5 g, L-arginine 4.9 g, L-cysteine 2.7 g, glycine 3.2 g, L-histidine 3.2 g, L-isoleucine 8.0 g, L-leucine 13.5 g, L-lysine 11.0 g, L-methionine 3.0 g, L-phenylalanine 5.7 g, L-proline 12.2 g, L-serine 6.9 g, L-threonine 6.9 g, L-tyrosine 6.9 g, and L-valine 8.9 g (Salomon, Miller, Krystal, Heninger, & Charney, 1997). Orange or chocolate mint flavoring was used to improve palatability of the drink. Cysteine, methionine, and arginine were encapsulated due to their unpleasant taste (Salomon et al., 2003). We used a 100 g drink rather than the 80 g drink used in some studies in order to produce the greatest effect. The 100 g drink was expected to produce ATD within 4.5–7 hr, with an 80–90% drop in plasma tryptophan and concomitant drop in central tryptophan (Salomon et al., 2003; Schmitt et al., 2006). The control drink was a 300 mL drink and encapsulated amino acids but in 1/4 strength: L-alanine (1.4 g), L-arginine (1.2 g), L-cysteine (0.7 g), glycine (0.8 g), L-histidine (0.8 g), L-isoleucine (2.0 g), L-leucine (3.4 g), L-lysine (2.8 g), L-methionine (0.8 g), L-phenylalanine (1.4 g), L-proline (3.1 g), L-serine (1.7 g), L-threonine (1.7 g), L-tyrosine (1.7 g), L-valine (2.2 g), and fillers (7.95 g; Booij et al., 2003). The control drink looked and tasted like the treatment drink to ensure blinding and was expected to lower tryptophan by only 25% (Carpenter et al., 2009; Salomon et al., 1997).

Procedure

Procedures for the 2 study weeks were identical. Participants arrived at the General Clinical Research Center after fasting for 8 hr. Outpatient admission procedures included obtaining a brief medical history, conducting a physical examination, collecting a urine sample for drug

screening, and placing an intravenous catheter with heparinized saline for blood draws. To obtain a baseline, we drew blood and asked participants to fill out demographic, symptom, and mood questionnaires. The study start (Time 0) coincided with ingestion of the amino acids. We completed subsequent blood draws hourly for 8 hr to monitor tryptophan, amino acids, and glucose levels. The neuropsychological assessment was completed 5 hr after amino acid ingestion, when tryptophan levels were expected to be at their nadir. Symptom and mood questionnaires were completed at 3, 5, and 7 hr. Participants engaged in quiet activities while being monitored by nurses. At 8 hr, a meal containing 0.25 g of tryptophan was served. The study psychologist verified absence of depressive symptoms, we removed the intravenous access, and women were discharged. The next morning, study staff called the subject to verify the absence of depression using the Hamilton Rating Scale-Depression (HRSD) and assess side effects using a checklist. The investigative team and a data safety monitoring board routinely reviewed all safety monitoring data.

Demographic and Medical Information

We used a questionnaire to collect the following demographic information: birth date, race, marital status, education, employment status, income, current medications, menopausal status, gynecological, and reproductive history. Participants self-reported breast cancer information, which study staff verified through medical record review (date of diagnosis, disease stage, and dates/types of treatments). Participants also completed the Comorbidity Questionnaire to document the presence of medical problems and whether the condition caused problems, required medication, and/or limited activities (Sangha, Stucki, Liang, Fossel, & Katz, 2003).

Mood

We used the HRSD (Hamilton, 1960) to rule out depressive symptoms at study entry, at the end of each test day, and the day after each test day (i.e., score ≥ 18). This scale has been used to monitor response in other ATD studies (Ruhé et al., 2007). Interrater reliability of HRSD administration by the study psychologist and the study nurse exceeded 90%. We also monitored mood throughout each test day (baseline and hours 3, 5, and 7) and the next morning using the Profile of Mood States-Short Form (POMS-SF), a 37-item scale that is based on the 65-item version (McNair, Lorr, & Droppelman, 1971; Shacham, 1983). This instrument has a list of adjectives that participants rate on a 0–4-point scale; responses are summed to generate a total score from six subscale scores (depression, tension, anger, confusion, vigor, and fatigue). The POM-SF scale has been used in other studies to monitor response to ATD (Ruhé et al., 2007), and its reliability and validity in women with breast cancer has been supported (Curran, Andrykowski, & Studts, 1995).

Glucose

We assessed serum glucose at each hourly blood draw (YSI Life Sciences Instrument, Yellow Springs, OH) and administered glucose tablets by mouth for values ≤ 70 mg/dL. We monitored glucose levels to prevent hypoglycemia and to ensure that changes in cognition between groups (ATD/control and control/ADT) were not due to hypoglycemia.

Neuropsychological Tests

A trained and certified research assistant administered the cognitive assessment battery at Hour 5. Alternate forms were used as available, and the battery took approximately 45 min to complete. The cognitive function tests chosen for this study are standardized and validated neuropsychological assessments (Lezak, Howieson, & Loring, 2004) and have been used with breast cancer patients (Von Ah, Harvison, et al., 2009).

Learning and memory—We used the RAVLT to test learning and verbal memory (Lezak et al., 2004). Participants were presented with a 15-item word list for 5 learning trials, and recall was taken immediately after each trial to determine immediate memory. A distracter word list was presented just prior to short-delayed free recall of the initial list. Long-delayed free recall of the initial word list was taken 30 min later. The score is the total number of words recalled.

Working memory—We used the Digit Span test (Wechsler, 1981) to assess working memory. Digit span forward requires participants to listen to a series of digits (from two to nine digits in length) and repeat them aloud in the order given. The digit span backward requires participants to listen to a series of digits (two to eight digits in length) and repeat them aloud in the reverse order. Higher numbers of digits repeated in the correct order indicate better working memory function.

Verbal fluency—We used Controlled Oral Word Association (COWA; Benton & Hamsher, 1989) to evaluate verbal fluency. The test has been reported to be highly sensitive to frontal lobe lesions. For this test, we asked participants to produce as many words beginning with a specified letter as they could in 60 s.

Information Processing Speed: The Symbol Digit Modalities Test (Smith, 1982) was used to measure processing speed. Participants were asked to substitute simple geometric shapes for numbers 1 to 9 using a legend.

Psychomotor ability (motor speed)—The finger tapping test is one of the most widely used measures of manual motor functioning (Reitan & Wolfson, 1993). Using a small lever attached to a board, the subject taps with her index finger as quickly as possible for 10 s. The counter records the number of taps. Three trials are given for each hand (dominant and nondominant). The score is the average of the three trials for each hand. This test takes approximately 3 min to complete.

Biochemical Measures

Laboratory assessments included (1) hourly measurements of circulating tryptophan during each test day and (2) assessment of tryptophan/LNAA (TRP/LNAA) ratios at the presumed 5-hr tryptophan nadir each week. We assayed whole blood tryptophan and hydroxyl-tryptophan with high-performance liquid chromatography (HPLC) using procedures similar to those described by others (Xiao, Beck, & Hjendahl, 1998). This assay can reproducibly quantify tryptophan concentrations $6.25 \mu\text{M}$, which is sufficient to detect the low levels we expected. LNAAs concentrations were determined by the Waters Pico-Tag methods. Briefly, the amino acids were derivitized with phenylisothiocyanate, forming phenylthiocarbonyl derivatives. The resulting samples were then analyzed by reversed phase HPLC separation and ultraviolet (UV) detections, using a Waters Alliance HPLC system.

Genetic Markers

Genetic polymorphisms in three serotonin-related candidate genes were assessed for association with cognitive functioning. We chose these specific polymorphisms because previous investigators have reported that they were associated with clinical phenotypes (Moreno et al., 2002). Procedures for genotyping used in this study have been described previously (Carpenter et al., 2009). Briefly, we genotyped the following single nucleotide polymorphisms: rs#6313 and rs#799701 in the serotonin receptor 2A gene (*HTR2A*); rs#1800532 from the tryptophan hydroxylase gene (*TPH1*); and rs#rs6295 from the serotonin receptor 1A (*HTR1A*) gene. DNA was extracted from whole blood using the Gentra DNA extraction kit. The HTR1A and HTR2A SNPs were genotyped using Taqman

assays from Applied Biosystems, Inc. (assay id#'s c__11904666_10 [rs6295]; c__3042197_1_ [rs6313]; and c__1619749_10 [rs7997012]). We conducted genotyping for the TPH1 SNP using an allele-specific polymerase chain reaction (PCR) assay as follows: we used the iCycler system (Bio-Rad) with allele-specific primers (common forward primer: 5'-AGA ATG GTA CCT GGC ATG AAA-3', reverse primer for the allele containing A: 5'-C CTA TGC TCA GAA TAG CAG CTC T-3', reverse primer for the allele containing C: 5'-CTA TGC TCA GAA TAG CAG CTC G-3') and with the SYBR green Super-mix (Bio-Rad). The allele-specific real-time PCR was run for 45 cycles at 95°C for 10 s and 55°C for 45 s. Alleles were discriminated based on their Ct values.

Statistical Analysis

We examined sample characteristics using descriptive statistics and compared subjects who received ATD/control and control/ATD using two-sided *t*-tests and Fisher's exact tests. We also used Fisher's exact tests to compare tryptophan values and TRP/LNAA ratios at the nadir time point (5 hr following drink ingestion) between depletion and control conditions to verify that adequate depletion was reached.

We used a separate mixed linear model to evaluate each of the dependent variables including memory (immediate and delayed recall), working memory, verbal fluency and executive functioning, speed of processing, and psychomotor ability. Carry-over effects were tested with order (ATD/control and control/ADT) effect, period (test day 1 vs. 2) effect, and their interaction effect using a mixed linear model. We found that mood and glucose levels at the nadir were not significantly correlated with any of the cognitive values and thus, did not retain them in the final models.

We also tested whether response to the ATD versus control conditions varied by the following: mood (total POM-SF), glucose levels, use of SSRI/SSNRI, use of antiestrogens (such as Tamoxifen or aromatase inhibitors), breast cancer disease and treatment variables (time since diagnosis, type of treatment), and genetic polymorphisms including HTR1A, HTR2A, and TPH1. Due to small sample sizes, genetic analyses were conducted by descriptive methods.

Results

The accrual flow of the study is shown in Figure 1. Of the 22 consenting women, one withdrew after Week 1, and due to interruptions during cognitive testing, we did not use one other participant's data. Among the remaining 20 women, 18 (90%) were Caucasian, 17 (85%) were married or living with a partner, and 14 (60%) had household incomes above \$60K. The mean age was 51.2 years (*SD* = 9.3, range 30–66). All had been successfully treated for nonmetastatic breast cancer (i.e., Stage IV or less) and were considered to be free of cancer at the time of the study. Mean time postcompletion of primary treatment (i.e., surgery, chemotherapy, radiation) was 32.8 months (*SD* = 14.3, range 13–57). Breast cancer treatments received included 3 (15%) with surgery alone, 1 (5%) with surgery with radiation therapy, 5 (25%) with surgery with chemotherapy, and 11 (55%) with surgery with radiation and chemotherapy. None of the women were taking hormone replacement therapy, 7 (35%) were taking tamoxifen, 9 (45%) were taking an aromatase inhibitor, and 8 (40%) were taking selective serotonin reuptake inhibitors/selective serotonin–norepinephrine reuptake inhibitors, a common treatment for hot flashes in this patient population. None of the women had a change in their medication during the 2 weeks of the study.

We found no significant differences between groups (referred to as an order effect: ATD/control vs. control/ATD) for age, race, marital status, education, employment, income, time

since diagnosis, type of breast cancer treatment, or use of SSRI/SSNRI, suggesting adequate balancing among possible confounding factors through randomization.

Biochemical Effect

Tryptophan values were not significantly different ($t = -0.70$, $df = 15$, $p = .50$) between groups at baseline. As anticipated, the active amino acid drink resulted in a statistically significant drop in tryptophan from +5 hr (nadir) compared with the control drink (see Figure 2). At +5 hr (nadir), the active amino acid drink resulted in an 85.4% drop in tryptophan, whereas the control drink resulted in a 49.3% decrease. Similarly, at +5 hr, TRP/LNAA ratios were significantly lower during ATD ($M = 0.0029$, $SD = 0.0025$) compared to control ($M = 0.02$, $SD = 0.01$; $p < .0001$). In addition, it should be noted that the variability in tryptophan at +5hr for the depletion arm was similar to those in other published reports using 100 g AA concentrations (Mendelsohn et al., 2009). Also, importantly, there was a clear separation between the depletion ranges for the treatment and control drinks at +5 hr (nadir) when the neuropsychological assessments were performed (see Figure 2).

The order of treatment (ATD/control vs. control/ATD) was not significant. However, the period effect (Week 1 vs. Week 2) had a significant main effect and was controlled for in each of the analyses. Table 1 displays the mean, standard deviation, and intervention effect for each dependent variable. ATD had a negative impact on both memory ($F[1, 17] = 4.86$, $p = .04$) and motor speed for the dominant hand ($F[1, 17] = 4.51$, $p = .04$) and for the nondominant hand ($F[1, 17] = 6.38$, $p = .02$; see Figure 3). In addition, there was a significant interaction effect of order of treatment and period for motor speed for the dominant hand ($F[1, 18] = 6.17$, $p = .02$), such that slower speed was associated with depletion in Week 1.

We also tested whether response to the ATD versus control condition varied by mood, glucose levels, or genetic polymorphisms. Total mood disturbance scores were similar over time ($F[4, 190] = 1.49$, $p > .20$) between the two groups (Figure 4A). Similarly, there were no differences in blood glucose over time ($F[6, 257] = 0.32$, $p > .20$; Figure 4B). These data suggest that the significant changes noted in memory and psychomotor ability were not influenced by mood or blood glucose levels. In addition, we noted no differences in response to ATD based on genetic variants in the *HTR1A*, *HTR21*, and *TRH1* genes.

Discussion

The primary aim of this study was to evaluate the role of lowered serotonin synthesis on neuropsychological function in female postmenopausal BCSs using ATD. A significant subpopulation of BCSs report cognitive deficits, which often correlate with depressive symptoms. To our knowledge, this was the first study to use these methods to examine cognitive functioning in BCSs. We hypothesized that ATD would negatively alter neuropsychological functioning in these female BCSs. Specifically, we hypothesized that deficits in central serotonin would induce cognitive deficits including decreases in immediate and delayed recall, working memory, verbal fluency, speed of processing, and psychomotor ability.

We found significant differences in performance on episodic memory (delayed recall) and motor speed during the depletion condition compared to the control condition. These effects were seen in the absence of deficits in immediate recall, working memory, verbal fluency, or information processing speed and could not be explained by fluctuating mood or blood glucose levels. Findings suggest that lowered central serotonin has selective effects on episodic memory, specifically delayed recall, and motor speed.

The finding of impairment in delayed recall as measured by the RAVLT is consistent with recent studies using ATD to examine neuropsychological functioning, specifically in individuals with “serotonergic vulnerability” (pooled sample of healthy elderly and recovered depressed patients and remitted depressed patients; Mendelsohn et al., 2009). These results suggest that ATD impairs memory formation, presumably as a result of an acute decrease in serotonin turnover in the brain. This decrement in memory (delayed recall) was independent of any effect on new learning (immediate recall) or working memory, suggesting that serotonin may interact with other systems in affecting consolidation or retention of information after learning (Schmitt et al., 2000).

While some ATD studies have found equivalent results for healthy controls, a recent comprehensive systematic review regarding the effects of ATD on episodic memory (specifically delayed recall) identified that ATD impacts vulnerable populations more profoundly. These authors concluded that “the effects of ATD seem more pronounced in the elderly, depressed, and individuals with the vulnerable serotonin transporter genotype” (Mendelsohn et al., 2009, p. 949). Taken together, our preliminary work and previous research suggest that BCSs may be more vulnerable to the effects of ATD on episodic memory functioning. However, further studies are warranted to determine whether the effects of ATD differ between postmenopausal BCSs and healthy menopausal women, providing a more powerful indication of serotonergic vulnerability in this population.

We also found that ATD impaired motor speed using the finger tapping test. Serotonin has been shown to modulate psychomotor ability (Tao & Auerbach, 2003), and thus, we hypothesized that the depletion would disrupt motor ability. Our finding of impairment in motor ability is supported by a previous study that found that citalopram (a selective serotonin reuptake inhibitor) improved psychomotor ability (Nathan, Sitaram, Stough, Silberstein, & Sali, 2000). However, we found only two studies that used ATD to explore the role of serotonin in motor ability, and results were not significant (Luciana, Burgund, Berman, & Hanson, 2001; Porter et al., 2003). Luciana and colleagues explored the impact of tryptophan depletion and tryptophan loading on psychomotor functioning including motor ability (using the finger tapping test) and accuracy (using grooved pegboard) in 19 healthy adults. While they noted no significant differences in motor ability or accuracy with acute depletion, they did find significant decrements in motor accuracy (dropped more pegs) during tryptophan loading. Similarly, in the second study, Porter and colleagues noted no significant impairment in a motor screening test in a group of individuals with Alzheimer’s disease or a healthy elderly group. Discrepancies in the findings between studies may be explained by differences in population and/or measurements used to examine motor ability. Further research is needed to explore the role of serotonin in psychomotor functioning specifically for BCSs. Future studies may benefit from using a more comprehensive approach to measuring psychomotor functioning by including tests that tap into perceptual functioning (reaction times) as well as motor abilities (speed and accuracy; Mendelsohn et al., 2009).

We noted no significant main effects of ATD on working memory, verbal fluency, or information processing speed. These findings are consistent with the majority of previous studies to date, although results have been mixed (Mendelsohn et al., 2009).

Secondarily, we attempted to address limitations of previous studies by controlling for the known confounding issues of mood and glucose level on cognitive performance. Mood and glucose levels did not correlate with cognitive functioning at the nadir on either test day nor did they differ significantly between treatment and control weeks.

We also explored whether differences in genetic variations in serotonin receptor and synthesis genes altered responses to ATD. We found no differences based on genetic variants in the *HTR1A*, *HTR21*, and *TRH1* genes. However, this study was not powered adequately for this end point, and the data should be regarded as exploratory only. While we acknowledge the limitations of these data, exploration of determinants of individual variability that may impact ATD is warranted.

Study findings must be considered in light of methodological considerations and study limitations. First, the type of design of this ATD study has significant implications. Similar to most of the ATD studies to date, we utilized a repeated measures crossover design to examine differences between treatment and control arms of the study (Mendelsohn et al., 2009). Advantages of using the repeated measures design in this ATD study included (1) the ability to control for known confounding issues of cognitive functioning (i.e., age, education, medication usage, etc.), (2) the requirement of fewer participants, and (3) increased power to detect effects. These considerations are particularly important when designing expensive laboratory studies (Von Ah & Carpenter, 2008). We were able to compare our study findings to pooled summaries of recent ATD studies. However, future work would benefit from including a comparison group of healthy menopausal women and conducting between-subjects analyses. These analyses may be able to offer more insight regarding the serotonergic vulnerability of this breast cancer population.

Second, a significant methodological consideration when conducting ADT studies is the amount of amino acid concentration used for both the intervention and placebo control arms to ensure adequate depletion and separation of depletion levels between treatment arms. In this study, we found that the differences in both TRP depletion and the TRP/LNAA ratios and the variation in depletion at the nadir (+5 hr) time point were equivalent to other published ATD studies. There was no overlap in depletion levels noted at the nadir (+5 hr) between treatment arms; overlap could have led to false negative findings. However, variation in depletion is an important aspect of these studies and more work needs to be done to identify individual variability (e.g., genotyping) that may alter ATD effects. We recognize that significant findings in all cognitive domains may have been difficult to detect in this study due to the 49% drop in tryptophan seen with the one-fourth strength control drink. More recent reports have found that the one-fourth control drink may deplete tryptophan to up to 50%. In fact, some studies have begun to label the 100 g amino acid concentration treatment drink and the one-fourth strength control drink as high- and low-dose tryptophan depletion due to their depletion effects, respectively. Alternatives for the control drink, however, are limited, as researchers seek to ensure that the taste and consistency of the amino acid drinks are comparable for adequate blinding of study participants. Future research may benefit from comparing tryptophan depletion and repletion paradigms on cognitive functioning as one alternative for alleviating this issue.

And finally, our sample size, though larger than two thirds of the neuropsychological studies highlighted in a recent meta-analysis (Sambeth et al., 2007), was limited in terms of racial and ethnic diversity.

In conclusion, this study provides initial evidence that lowered serotonin levels via ATD specifically interfere with episodic memory (delayed recall) and motor speed in postmenopausal BCSs. The ATD cognitive effects we found were independent of changes in mood or blood glucose levels. Overall, this preliminary work is an important and necessary first step in understanding the role of serotonin in cognitive functioning in this potentially vulnerable population. In addition, this line of research has great potential to broaden our understanding of the underlying mechanisms involved with cognitive deficits in BCSs and to offer insights for the development of innovative and novel treatments.

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Figure 1.

Study accrual and retention. This figure details the study accrual, retention, and attrition from the time patients were approached in the clinic, screened for eligibility, expressed interest and consented, and then completed both weeks of study. Data were dropped for one subject due to environmental issues that interfered with the cognitive testing.

Figure 2. Percentage change in serum tryptophan over time during acute tryptophan depletion (ATD) versus control arms. This figure denotes the mean percentage change in tryptophan with standard deviation bars from baseline over time for ATD (solid line with diamonds) compared to control condition (dashed line with squares) for subjects ($n = 20$). Times 0 to 8 refer to hourly blood draws during each test day. Tryptophan values were not significantly different ($p = .46$) between randomized groups at baseline, but at the 5-hr nadir when cognitive testing occurred, they had decreased significantly in both conditions and were significantly lower in the ATD condition than in the control condition.

Figure 3. (A) Rey AVLT-delayed recall (memory; top graph) and (B) psychomotor ability (motor speed) for dominant and nondominant hand (bottom graph) by group interaction.

Figure 4.

Mean score total mood disturbance and mean glucose level. This figure denotes (A) the mean percentage change in mood disturbance and (B) glucose levels from baseline over time for acute tryptophan depletion (solid line with diamonds) compared to control condition (dashed line with squares) for subjects ($n = 20$). Mood disturbance was graphed for times 0, 3, 5, and 7 hr and the next day. Blood glucose levels were measured every hr from times 0 to 8 during each test day. Mood disturbance and blood glucose level did not significantly differ between ADT and control conditions ($p = .21$ and $.93$, respectively).

Table 1

Mean (Standard Error) of Cognitive Function Test Scores (Dependent Variables) During Acute Tryptophan Depletion Versus Control Conditions

Cognitive Function Test	Acute Tryptophan Depletion	Control	p^*
Learning: AVLT sum recall	53.64 (1.85)	52.81 (1.89)	.65
Memory: AVLT delayed recall	9.87 (0.57)	10.77 (0.58)	.04
Attention: Digit span	18.34 (0.87)	17.41 (0.88)	.18
Information processing speed: Symbol digit substitution	57.27 (2.80)	56.96 (2.83)	.86
Verbal fluency: COWA	43.09 (2.82)	40.83 (2.86)	.29
Motor functioning, dominant hand: Finger tapping test	124.47 (4.04)	130.27 (4.10)	.04
Motor functioning, nondominant hand: Finger tapping test	120.59 (3.58)	127.17 (3.62)	.02

Note. AVLT = auditory verbal learning test; COWA = controlled oral word association.

* p value is F test of intervention effect.