



Københavns Universitet

Neuroticism Associates with Cerebral in Vivo Serotonin Transporter Binding Differently in Males and Females

Tuominen, Lauri; Miettunen, Jouko; Cannon, Dara M; Drevets, Wayne C; Frokjaer, Vibe G; Hirvonen, Jussi; Ichise, Masanori; Jensen, Peter S; Keltikangas-Järvinen, Liisa; Klaver, Jacqueline M; Knudsen, Gitte Moos; Takano, Akihiro; Suhara, Tetsuya; Hietala, Jarmo

Published in:

International Journal of Neuropsychopharmacology

DOI:

[10.1093/ijnp/pyx071](https://doi.org/10.1093/ijnp/pyx071)

Publication date:

2017

Citation for published version (APA):

Tuominen, L., Miettunen, J., Cannon, D. M., Drevets, W. C., Frokjaer, V. G., Hirvonen, J., ... Hietala, J. (2017). Neuroticism Associates with Cerebral in Vivo Serotonin Transporter Binding Differently in Males and Females. *International Journal of Neuropsychopharmacology*, 20(12), 963-970. <https://doi.org/10.1093/ijnp/pyx071>

REGULAR RESEARCH ARTICLE

Neuroticism Associates with Cerebral in Vivo Serotonin Transporter Binding Differently in Males and Females

Lauri Tuominen, MD, PhD; Jouko Miettunen, PhD; Dara M. Cannon, PhD; Wayne C Drevets, MD; Vibe G. Frokjaer, MD, PhD; Jussi Hirvonen, MD, PhD; Masanori Ichise, MD, PhD; Peter S. Jensen, MSc; Liisa Keltikangas-Järvinen, PhD; Jacqueline M. Klaver, PhD; Gitte M. Knudsen, MD, PhD; Akihiro Takano, MD, PhD; Tetsuya Suhara, MD, PhD; Jarmo Hietala, MD, PhD

Turku PET Centre, Turku University Hospital, Turku, Finland (Drs Tuominen, Hirvonen, and Hietala); Department of Psychiatry, Massachusetts General Hospital and Harvard Medical School, Cambridge, MA (Dr Tuominen); Center for Life Course Health Research, University of Oulu, Finland & Medical Research Center (MRC) Oulu, Oulu University Hospital and University of Oulu, Oulu, Finland (Dr Miettunen); Centre for Neuroimaging & Cognitive Genomics (NICOG), Clinical Neuroimaging Laboratory, NCBES Galway Neuroscience Centre, College of Medicine Nursing and Health Sciences, National University of Ireland, Galway, Ireland (Dr Cannon); Janssen Research & Development, LLC, of Johnson & Johnson, Titusville, NJ (Dr Drevets); Neurobiology Research Unit, Rigshospitalet, Denmark (Dr Knudsen); Center for Integrated Molecular Brain Imaging, Rigshospitalet, Denmark (Dr Frokjaer and Mr Jensen); Department of Radiology, University of Turku, Turku, Finland (Dr Hirvonen); Department of Functional Brain Imaging Research, National Institute of Radiological Sciences, National Institute for Quantum and Radiological Science and Technology, Chiba, Japan (Drs Ichise, Takano, and Suhara); IBS, Unit of Personality, Work and Health Psychology, University of Helsinki, Helsinki, Finland (Dr Keltikangas-Järvinen); Department of Psychology, Southern Illinois University, Carbondale, Illinois (Dr Klaver); Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark (Dr Knudsen); Center for Psychiatric Research, Department of Clinical Neuroscience, Karolinska Institutet, Stockholm, Sweden (Dr Takano); Department of Psychiatry, University of Turku, Turku, Finland (Dr Hietala).

Correspondence: Lauri Tuominen, MD, PhD, MGH/HST Athinoula A. Martinos Center for Biomedical Imaging, 149 13th St, Charlestown, MA 02129 (ltuominen@mgh.harvard.edu).

Abstract

Background: Neuroticism is a major risk factor for affective disorders. This personality trait has been hypothesized to associate with synaptic availability of the serotonin transporter, which critically controls serotonergic tone in the brain. However, earlier studies linking neuroticism and serotonin transporter have failed to produce converging findings. Because sex affects both the serotonergic system and the risk that neuroticism poses to the individual, sex may modify

Received: April 10, 2017; Revised: June 16, 2017; Accepted: August 3, 2017

© The Author(s) 2017. Published by Oxford University Press on behalf of CINP.

This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/4.0/>), which permits non-commercial re-use, distribution, and reproduction in any medium, provided the original work is properly cited. For commercial re-use, please contact journals.permissions@oup.com

Significance Statement

Neurobiological underpinnings of the personality trait neuroticism are still not well understood. Here, using a large sample, we show that higher neuroticism associates with higher thalamic serotonin transporter binding in males, whereas in females, higher neuroticism associates with lower thalamic serotonin transporter binding. The finding helps to elucidate the brain-level molecular mechanisms predisposing to affective disorders. The study also provides clues into the neural mechanisms of sex differences in many psychiatric illnesses.

the association between neuroticism and serotonin transporter, but this question has not been investigated by previous studies.

Methods: Here, we combined data from 4 different positron emission tomography imaging centers to address whether neuroticism is related to serotonin transporter binding in vivo. The data set included serotonin transporter binding potential values from the thalamus and striatum and personality scores from 91 healthy males and 56 healthy females. We specifically tested if the association between neuroticism and serotonin transporter is different in females and males.

Results: We found that neuroticism and thalamic serotonin transporter binding potentials were associated in both males and females, but with opposite directionality. Higher neuroticism associated with higher serotonin transporter binding potential in males (standardized beta 0.292, $P = .008$), whereas in females, higher neuroticism associated with lower serotonin transporter binding potential (standardized beta -0.288, $P = .014$).

Conclusions: The finding is in agreement with recent studies showing that the serotonergic system is involved in affective disorders differently in males and females and suggests that contribution of thalamic serotonin transporter to the risk of affective disorders depends on sex.

Keywords: neuroticism, sex, serotonin, serotonin transporter, PET

Introduction

Women have higher incidence of major depressive disorder (MDD) and subclinical depressive symptoms than men (Lindeman et al., 2000). The neurotransmitter mechanisms that might contribute to this difference are still not well understood. On average women score higher in neuroticism, which is a personality trait that reflects the propensity for experiencing negatively valenced emotional states. By definition, individuals on the higher end of the neuroticism spectrum are more prone to feel negative emotions such as sadness and anxiousness and also have more difficulties when trying to cope with stress (Costa and McCrae, 1992a). Higher neuroticism scores also increase the risk for developing MDD (Kendler et al., 2006) and anxiety disorders (Hettema et al., 2006). Thus, sex differences in manifesting this trait may contribute to the sex difference in the incidence of MDD. Moreover, high neuroticism also increases the risk for MDD more so in women than in men (Kendler and Gardner, 2014), which suggests that sex impacts the mechanism through which neuroticism confers greater risk for affective disorders.

The sex difference in the incidence of MDD putatively is mediated at least partly by the effects of sex hormones, because the MDD incidence is higher in women only after puberty (Wade et al., 2002). Further, sex hormones affect the serotonin system in multiple ways (Barth et al., 2015), which may be one mechanism conveying the effects of sex on the incidence of affective disorder. Serotonergic function has a well-documented role in the pathophysiology of MDD and the mechanisms of antidepressant pharmacotherapy (Cannon et al., 2007; Meyer, 2007; Sharp and Cowen, 2011). The synaptic serotonin levels are crucially controlled by the serotonin transporter (5-HTT). Thus, the 5-HTT has been viewed as a promising target in the search for the neural underpinnings of neuroticism. Investigating the central 5-HTT in neuroticism may elucidate the neurobiological mechanisms of the greater susceptibility for developing

MDD and thereby illuminate the etiology of affective disorders (Marcus et al., 2008).

Despite initially promising findings, human studies linking 5-HTT and neuroticism have been inconsistent, and the role of 5-HTT in neuroticism has remained elusive. The S-allele in the 5-HTT gene-linked promoter region (5-HTTLPR) was initially found to be associated with higher neuroticism (Lesch et al., 1996). However, subsequently several negative findings have been published, and the latest meta-analyses have yielded inconclusive results, mainly due to the large heterogeneity in the results across studies (Munafo et al., 2009; Minelli et al., 2011). Intriguingly, recent evidence converges to suggest that sex may modulate the effects of 5-HTTLPR polymorphism on phenotype (Gressier et al., 2016). That is, in females, the S-allele seems to associate more with depression and negative affect, whereas in males it associates more with aggressive and externalizing traits.

So far, 3 published positron emission tomography (PET) studies have investigated whether neuroticism associates with in vivo 5-HTT binding. Takano and colleagues (Takano et al., 2007) found a positive association between neuroticism and thalamic 5-HTT binding, Klaver et al. (Klaver et al., 2007) a negative relationship, while Kalbitzer and colleagues (Kalbitzer et al., 2009) did not detect any significant relationship between these 2 measures. One explanation may be that single site PET studies often lack power due to too-small sample sizes. Moreover, the first study included only males, whereas the latter ones included both males and females. To date, no study has directly investigated whether sex modulates the effect of neuroticism on 5-HTT binding. If such a sex effect exists, it might also explain inconsistencies in the literature and elucidate the neurobiological basis for the greater incidence of MDD in women and the tendency for women to manifest a partly distinct symptom profile than men during major depressive episodes (Martin et al., 2013).

In the present study, we sought to resolve these conflicts in previous PET imaging studies regarding the association between 5-HTT and neuroticism. We combined data from 4 different imaging centers that have measured the 5-factor model personality questionnaire and 5-HTT binding using PET with a selective 5-HTT radiotracer. Importantly, the combined data set was large enough to allow us to test whether sex modulates the association between neuroticism on 5-HTT binding. Because previous studies have found correlations between other personality traits and 5-HTT (Kalbitzer et al., 2009), we also explored these associations in this data set.

Methods

Study Design

The study sample comprises 147 healthy subjects (91 males and 56 females) from 4 independent imaging centers (Table 1). The imaging centers were: the National Institute of Radiological Sciences, Chiba, Japan (NIRS), the Neurobiology Research Unit, Copenhagen University Hospital Rigshospitalet, Denmark (NRU), the National Institute of Mental Health, Bethesda, Maryland, USA (NIMH), and the Turku PET Centre, Turku, Finland (TPC). NIRS, NRU, and NIMH have previously published these data (Klaver et al., 2007; Takano et al., 2007; Kalbitzer et al., 2009; Erritzoe et al., 2010). In addition, unpublished data from 31 subjects were collected at the TPC, following the same methods described in Tuominen et al. (Tuominen et al., 2012). The protocols of the original studies were approved by local ethics committees, and subjects gave informed consent accepted by those committees. The study at the NIRS was approved by the Ethics and Radiation Safety Committee of the National Institute of Radiological Sciences, Chiba, Japan; the study at the NRU was approved by the Ethics Committee of Copenhagen and Frederiksberg, Denmark; the study at the NIMH was approved by the Institutional Review Board at the National Institute of Mental Health, Bethesda, MD; and the study at the TPC was approved by the by the Joint Ethical Committee of the University of Turku and the Turku University Central Hospital, Turku, Finland. At the NIH and Turku PET Centre, subjects were interviewed using the Structured Clinical Interview for DSM-IV, whereas at the NIRS and NRU the subjects underwent an unstructured interview. At all study sites, exclusion criteria included lifetime psychiatric illness and present substance abuse. At the TPC, serotonin transporter binding was measured using [¹¹C]MADAM (Lundberg et al., 2005) and personality traits using NEO-FFI (Costa and McCrae, 1992b), whereas other centers used [¹¹C]DASB (Houle et al., 2000) and NEO-PI-R

scale (Costa and McCrae, 1992a). To account for the different number of items in the NEO-PI-R and NEO-FFI, we calculated a mean neuroticism score for each individual by dividing the raw score by the number of items in the neuroticism scale. Thus, neuroticism scores ranged from 0 to 4 (each item is rated from 0 to 4). The PET data were collected with Siemens ECAT47 at the NIRS, with HRRT scanner (Siemens) at the TPC, and with GE-Advance scanner (General Electric) at the NRU and NIH. The data were modeled at the TPC using the simplified reference tissue model (Lammertsma and Hume, 1996) and at other centers using the multi-linear reference tissue model 2 (Ichise et al., 2003) with cerebellar gray matter as the reference region. These models allow the estimation of the nondisplaceable binding potential (BP_{ND} ; Innis et al., 2007), which equals $f_{ND} B_{avail}/K_D$, where f_{ND} is the free fraction of ligand in the nondisplaceable tissue compartment, B_{avail} is concentration of available receptors, and K_D is radioligand equilibrium dissociation constant. BP_{ND} is directly proportional to the density of 5-HTTs and therefore can be used as an index of their density. All the imaging data included into the present study were preprocessed and modeled at the respective center with the primary study in mind. The current analyses were performed using only the regional BP_{ND} values that were available. Voxel-wise BP_{ND} maps were not available.

At the NRU, regions-of-interest (ROIs) were delineated automatically (Svarer et al., 2005), whereas in other centers ROIs were delineated manually onto individual PET scans using the co-registered T1-weighted MRI scan. All 4 centers had delineated thalamus as an ROI, but otherwise the centers used different ROIs (supplementary Table 1). At the NIRS, the striatum was delineated as one ROI, whereas in the other 3 centers this ROI was divided into caudate and putamen. Because serotonin transporter binding in the caudate and putamen is highly correlated (Tuominen et al., 2014) and we did not have the sizes of each individual's ROIs, we computed an arithmetic mean of BP_{ND} s in the caudate and putamen and used that as an estimate of striatal BP_{ND} for the data from 3 centers that had delineated these regions separately. No other region in the brain was consistently defined by all the centers, and therefore the statistical analyses were confined to these 2 regions: the thalamus and striatum.

Statistical Methods

To better assess the effects of sex and age on the association between neuroticism and 5-HTT BP_{ND} s, individual data points were included into the statistical model instead of carrying out

Table 1. Details of the Data Collected at Each Center

Center	N (M/F)	Age	Neuroticism ^a	Questionnaire	Tracer	Serotonin transporter BP_{ND}	
						Thalamus	Striatum
NIRS ¹	31 (31/0)	23.6 ± 2.8	2.1 ± 0.46	NEO-PI-R	[¹¹ C]DASB	1.8 ± 0.29	1.3 ± 0.16
NRU ²	57 (37/20)	35.1 ± 18.0	1.5 ± 0.40	NEO-PI-R	[¹¹ C]DASB	1.8 ± 0.25	1.6 ± 0.18 ^b
NIMH ³	28 (8/20)	36.3 ± 9.1	1.9 ± 0.14	NEO-PI-R	[¹¹ C]DASB	1.7 ± 0.23	1.3 ± 0.17 ^b
TPC ⁴	31 (15/16)	39.1 ± 5.1	1.2 ± 0.76	NEO-FFI	[¹¹ C]MADAM	1.4 ± 0.15	1.1 ± 0.16 ^b

Values are presented as mean ± SD.

^aNeuroticism score ranges from 0 to 4 (each item is rated from 0 to 4) and was calculated by dividing the raw score by the number of items in the questionnaire.

^bArithmetic mean of nondisplaceable binding potential (BP_{ND} s) in the caudatus and putamen. 1, National Institute of Radiological Sciences, Chiba, Japan; 2, Neurobiology Research Unit, Copenhagen University Hospital Rigshospitalet, Denmark; 3, National Institute of Mental Health, Bethesda, MD; 4, Turku PET Centre, Turku, Finland.

a meta-analysis. Multiple linear regression analyses using the individual data were performed for the 2 brain regions (thalamus and striatum) separately. The 5-HTT BP_{ND} was used as the dependent variable, and the neuroticism*sex interaction term, neuroticism, sex, and age as independent variables. The different centers were coded as dummy variables and included as covariate in all statistical models. The interaction term was created by multiplying sex and neuroticism values. We predicted that the interaction term would significantly associate with 5-HTT BP_{ND} . To explore if data from a single center dominated the effect, we also carried out this analysis 4 other times, leaving one center at the time out from the analysis. To obtain estimates of standardized beta coefficients for males and females separately, multiple regression analyses were performed separately for both sexes with the 5-HTT BP_{ND} as the dependent variable, and neuroticism, age, and centers as independent variables.

To illustrate the results in the thalamus, a partial regression plot (Larsen and McCleary 1972) between neuroticism scores and the thalamic 5-HTT BP_{ND} values was created. First, we computed residuals for both sexes by regressing the 5-HTT BP_{ND} against the age and centers while omitting neuroticism scores from the model. Second, we computed residuals for both sexes by regressing neuroticism against the age and centers while omitting 5-HTT BP_{ND} values from the model. The resulting residual vectors can be understood as mean centered neuroticism scores corrected for the age and centers, and mean centered 5-HTT BP_{ND} values corrected for the age and center respectively. Finally, the residual vectors for both sexes from the above 2 regression analyses were plotted against each other.

Because previous studies have reported associations between other personality traits and serotonin transporter BP_{ND} (Kalbitzer et al., 2009), we also performed exploratory multiple linear regression analyses for the other 4 personality traits included in the inventory: extraversion, agreeableness, openness to experience, and conscientiousness. Despite some reports of associations between these personality traits and the serotonin system, there are no compelling theoretical reasons to assume that these personality traits are associated with the serotonin system. Thus, these analyses were considered as exploratory. In a similar manner, the model included 5-HTT BP_{ND} as the dependent variable, and personality trait, sex, age, and the centers as independent variables. As we did not have a priori hypothesis about sex interaction for other personality traits, the interaction term was not included into the analyses.

Results

Neuroticism and 5-HTT in the Thalamus

A statistically significant multiple linear regression was found using the thalamic BP_{ND} values as the dependent variable and neuroticism, neuroticism * sex, sex, age, and centers as independent variables ($F_{(7,139)} = 14.566, P < .001, R^2 = .394$). The 5-HTT BP_{ND} in the thalamus was significantly associated with the neuroticism*sex interaction term ($t = 2.749$, standardized $\beta = 0.963, P = .007$) and with the age term ($t = -2.542$, standardized $\beta = -.288, P = .014$). The multiple linear regression analyses for each sex separately using the thalamic BP_{ND} values as the dependent variable and neuroticism, age, and centers as independent variables were also significant (males: $F_{(5,85)} = 11.385, P < .001, R^2 = .366$; females: $F_{(4,51)} = 13.011, P < .001, R^2 = .466$). In males, neuroticism was a significant positive predictor of thalamic 5-HTT BP_{ND} ($t = 2.707$, standardized $\beta = .292, P = .008$), whereas in females, neuroticism was a significant negative predictor and a

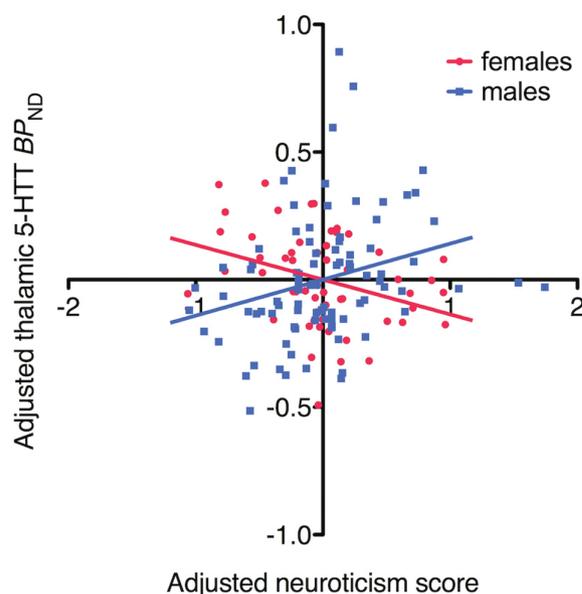


Figure 1. Partial regression plot illustrates the associations between neuroticism score and the thalamic serotonin transporter binding potential (5-HTT BP_{ND}) in males and females. Adjusted neuroticism scores are the residuals when regressing the neuroticism scores against age and center while omitting 5-HTT BP_{ND} values. Adjusted thalamic 5-HTT BP_{ND} values are the residuals when regressing BP_{ND} values against age and center while omitting neuroticism scores. Thus, the adjusted scores can be viewed as mean centered neuroticism scores and BP_{ND} values that have been corrected for age and study center.

negative association ($t = -2.542$, standardized $\beta = -.288, P = .014$). As illustrated in the partial regression plot (Figure 1), higher neuroticism scores in males were associated with higher 5-HTT BP_{ND} values in the thalamus, whereas higher neuroticism scores in females were associated with lower BP_{ND} values in the same region. Results from excluding one center at a time from the analyses are shown in supplementary Material and supplementary Figure 1.

Neuroticism and 5-HTT in the Striatum

The multiple linear regression model using the striatum BP_{ND} as the dependent variable and neuroticism, neuroticism * sex, sex, age, and centers as independent variables was statistically significant ($F_{(7,139)} = 20.022, P < .001, R^2 = .477$). However, no significant main effect of neuroticism or sex by neuroticism interaction was observed on the striatal 5-HTT BP_{ND} .

Extraversion, Agreeableness, Openness to Experience, and Conscientiousness and 5-HTT

Multiple regression analyses, using the other 4 personality traits (extraversion, agreeableness, openness to experience, and conscientiousness) were also carried out. However, in the resulting regressions models, none of these other personality traits were statistically significant predictors of the 5-HTT BP_{ND} in the thalamus or striatum (all $P > .05$).

Conclusions

The present study is the largest PET study to date that has examined the in vivo molecular brain biology basis of personality. The multi-site approach allowed us for the first time to show

that neuroticism and 5-HTT are linked in a sex-dependent manner. More specifically, in males, higher neuroticism scores associated with higher thalamic 5-HTT binding, whereas in females, high neuroticism scores associated with lower 5-HTT binding in the thalamus. In contrast, other personality traits described by the 5-factor model were not significantly associated with 5-HTT binding.

The interpretation between the magnitude of 5-HTT binding and intrasynaptic 5-HT levels is complex. Under some experimental conditions, higher 5-HTT binding correlates with decreased serotonin metabolite concentrations in the cerebrospinal fluid (Heinz et al., 1998, 2002). Nevertheless, the cell surface expression of 5-HTT sites generally parallels serotonin levels, as the cell surface expression of 5-HTT sites rapidly responds to changing serotonin concentrations (Blakely et al., 1998). Furthermore, the extent to which 5-HTT binding simply reflects the number of serotonergic afferents to projection areas is unknown. Thus, our data may not support simplistic hypotheses regarding the relationship between neuroticism ratings and synaptic serotonin levels per se.

An increasing body of literature supports the involvement of the cerebral serotonin system in neuroticism. Frokjaer and colleagues (Frokjaer et al., 2008) showed that higher neuroticism scores associate with higher 5-HT_{2A} receptor binding in the frontolimbic regions (n=83). More recently, we found that both cortical and subcortical 5-HT_{1A} receptor binding is lower in subjects who have higher neuroticism scores (Hirvonen et al., 2015) (n=34). Decreased 5-HT_{1A} and increased 5-HT_{2A} receptor binding in subjects with high neuroticism could result as compensatory changes to low serotonin levels (Hirvonen et al., 2015), which in turn have been linked to negative emotionality and depressive mood. For instance, low serotonin levels are associated with a negative emotional processing bias including an increase in the responsiveness to punishment (Cools et al., 2008; Fisher et al., 2015) and, in susceptible subjects, depressive symptoms (Benkelfat et al., 1994). In summary, the literature suggests that lower synaptic serotonin levels underlie higher neuroticism scores. Given the present results, we speculate that sex also affects the association between neuroticism and other constituents of the serotonin system. In this sample, we did not find a difference in 5-HTT BP_{ND} in the thalamus or striatum between males and females, but there is some evidence that healthy women have higher 5-HT_{1A} binding (Parsey et al., 2002) and lower serotonin synthesis rate (Nishizawa et al., 1997; Sakai et al., 2006) than males. Whether neuroticism correlates with serotonin synthesis rate or 5-HT_{1A} binding in a sex-dependent manner is currently unknown. Such an interaction could potentially have opposing effects with the present finding on the intrasynaptic serotonin levels and could help explain why 5-HTT binding associates with neuroticism differently in men and women. Despite that the exact molecular interpretation of our finding remains elusive, it clearly demonstrates that the underlying neurobiology of neuroticism is at least, to some extent, different between the sexes.

We found that neuroticism was associated with 5-HTT binding in a sex-dependent manner in the thalamus but not in the striatum. The mediadorsal and periventricular nuclei of the thalamus form part of the extended medial prefrontal network, which is centrally involved in MDD (Price and Drevets, 2010). Within that network, these thalamic nuclei putatively process, gate, and relay information from subcortical structures to the prefrontal cortex. Disrupting thalamic function by lesion leads to higher acute and chronic stress responses (Bhatnagar et al., 2002; Spencer et al., 2004), demonstrating its role in stress regulation.

In MDD patients, thalamic metabolism is increased (Price and Drevets, 2010; Su et al., 2014), but whether the thalamic 5-HTT binding is different in MDD patients has remained elusive. Previous PET studies have found both increased and decreased 5-HTT binding in the thalamus in MDD patients (Savitz and Drevets, 2013). Similarly, the literature is mixed on whether MDD associates with increases or decreases in 5-HT_{1A} and 5-HT_{2A} receptors (Shrestha et al., 2012; Savitz and Drevets, 2013).

Unfortunately, psychiatric imaging studies have rarely taken into account the possible modifying effects of sex. The few studies that have done so suggest that there may be important sex differences in the neurobiology of affective disorders. Recent data has shown that patients with seasonal affective disorder have upregulated cerebral 5-HTT in the winter, whereas individuals that are resilient to the disorder have downregulated 5-HTT (Mc Mahon et al., 2016). This result was mostly driven by the female participants. The sex-specific differences may also apply to other parts of the serotonin system. Serotonin synthesis capacity may be higher in female MDD patients than in male patients (Frey et al., 2010). On the other hand, one study reported that male MDD patients have elevated 5-HT_{1A} receptor levels but females do not (Kaufman et al., 2015). A similar sex difference has been shown in patients with panic disorder: male patients have higher 5-HTT levels than male controls, whereas 5-HTT binding did not significantly differ between female patients and female controls (Maron et al., 2011; Cannon et al., 2013). Our results support putative sex differences in the neurobiology of affective disorders. Because neuroticism is a major risk factor for affective disorders, our results further suggest that sex differences are critical in the brain architecture of risk for MDD. Indeed, even the 5-HTTLPR polymorphism may affect males and females differently. Gressier and colleagues (Gressier et al., 2016) showed that females suffering from MDD more often had the S-allele, whereas males with MDD more often had the L-allele. The S-allele leads to a lower number of 5-HTT proteins, which is detected as lower binding in PET studies (Willeit and Praschak-Rieder, 2010). Neuroticism can be viewed as an intermediary endophenotype between genetic predisposition and an affective disorder. The present study further demonstrates this endophenotype has different neurobiological correlates of risk for affective disorders in males and females.

Limitations

Results from this study should be appraised in the context of a number of limitations. First, women experience more subclinical depressive symptoms compared with men (Lindeman et al., 2000). Especially in a cross-sectional setting, it is difficult to disentangle high neuroticism personality from subclinical depression. Thus, it is possible that the negative association in females is partially mediated by subclinical depressive symptoms rather than associated with neuroticism personality trait per se. Second, we did not measure the phase of the menstrual cycle in females nor did we have sex steroid hormone levels available for the females. Although sex steroid hormones affect the 5-HT_{2A} receptor density (Moses et al., 2000; Kugaya et al., 2003; Moses-Kolko et al., 2003) and high-dose sex steroid hormones also affect the 5-HTT binding (Kranz et al., 2015), differences in the phase of the menstrual cycle are unlikely to contribute to the finding in females as the phase does not affect 5-HTT binding (Jovanovic et al., 2009; Frokjaer et al., 2015).

Another limitation is that individual body mass index (BMI) values were not available, and therefore we did not adjust the regressions for BMI. Erritzoe and colleagues showed an inverse association between BMI and 5-HTT binding (Erritzoe et al.,

2010). Because higher neuroticism typically associates with higher BMI (Vainik et al., 2013), spurious variations in BMI could not explain the positive association seen in males, but such variations could potentially contribute to the negative association seen in females. Finally, the amount of daylight has an effect on 5-HTT BP_{ND} (Praschak-Rieder et al., 2008), and this effect may depend on 5-HTTLPR status (Kalbitzer et al., 2010), which in itself has a moderate effect on 5-HTT BP_{ND} (Savitz and Drevets, 2013). In the present study, neither 5-HTTLPR status nor seasonal variations were controlled for.

Combining data from 4 independent centers also inherently introduces methodological discrepancies. In this study, a number of potentially confounding methodological differences were present between centers. First, neuroticism was measured using NEO-PI-R in all centers except one, where a shorter version of the NEO-PI, called NEO-FFI, was used. Because correlation between NEO-PI-R and NEO-FFI neuroticism scores ranges between 0.89 and 0.92 (Costa and McCrae, 1992b), differences between these instruments are unlikely to affect the results. Second, different centers used different PET scanners, which may have affected the 5-HTT BP_{ND} estimates. Further, the ROI's were delineated differently across the centers. The thalamus and striatum are easily distinguishable from the PET and T1-weighted MRI and are therefore easy to delineate. However, as the 5-HTT BP_{ND} is not uniform even within these structures, differences in the delineation procedures may have introduced some error variance into the BP_{ND} estimates. Moreover, 5-HTT binding was measured with 2 different tracers, [^{11}C]MADAM and [^{11}C]DASB. Both tracers bind specifically to the 5-HTT (Emond et al., 2002; Wilson et al., 2002), and the rank values and test-retest variability of different regions are highly comparable (Kim et al., 2006; Lundberg et al., 2006). There are no published head-to-head comparisons between the 2 tracers, but [^{11}C]MADAM seems to give somewhat lower BP_{ND} values, at least in the regions included. However, systematically lower 5-HTT binding values in a single sample are unlikely to affect the overall finding, because centers effects were statistically adjusted for. Additionally, we tested whether our finding would remain the same if the centers would be excluded, one at a time, from the analysis. These analyses showed that excluding the study that used [^{11}C]MADAM made the neuroticism by sex interaction an even more significant predictor of the 5-HTT BP_{ND} in the model. Surprisingly, after excluding the NRU sample, the interaction effect was no more statistically significant. This may be simply due to the fact that the NRU sample is the largest sample, and excluding it reduced the power of our analysis. Nonetheless, these analyses highlight the importance of large data sets to discover associations between behavior and brain neurotransmitter systems. Finally, in our study we did not look for associations beyond thalamus and striatum, as no other regions were consistently delineated by all the centers. Moreover, the delineation methods were likely to be more variable across the centers for other regions compared with the thalamus and striatum, which are easily distinguishable from the PET and T1-weighted MRI. Therefore, this combined data set cannot assess if the difference in the neuroticism by sex interaction in the 5-HTT BP_{ND} extends beyond the thalamus and striatum. Future studies must elucidate if the results extend to other brain regions.

In summary, we found that in males, neuroticism associates positively with 5-HTT binding in the thalamus, whereas in females the association was negative. Our results bridge the gap between genetic studies and clinical PET imaging studies that both have shown evidence for sex differences in how the serotonin system is involved in the pathophysiology of affective

disorders. This finding underscores the importance of taking sex into account when studying normal brain function. Better understanding of sex effects will hopefully facilitate better understanding of the etiology of psychiatric disorders. Since many published single-site PET studies lack power due to too-small sample sizes, the approach to combine many data sets applied here should be encouraged in the molecular imaging field. In summary, our results suggest that thalamic 5-HTT could contribute differently to the risk of affective disorders in males and females. Future studies need to elucidate if similar sex differences exist in other constituents of the serotonin system.

Acknowledgments

The authors thank Synthia Guimond and Lauri Nummenmaa for their helpful comments on the manuscript.

Statement of Interest

Wayne C. Drevets is an employee of Janssen Research & Development, LLC, of Johnson & Johnson, Inc. Lauri Tuominen is supported by a Sigrid Juselius Fellowship grant. Neither Johnson & Johnson, Inc. nor Sigrid Juselius Foundation influenced data collection, analysis, or interpretation of the results in any way. None of the other authors declare conflicts of interests.

References

- Barth C, Villringer A, Sacher J (2015) Sex hormones affect neurotransmitters and shape the adult female brain during hormonal transition periods. *Front Neurosci* 9:37.
- Benkelfat C, Ellenbogen MA, Dean P, Palmour RM, Young SN (1994) Mood-lowering effect of tryptophan depletion. Enhanced susceptibility in young men at genetic risk for major affective disorders. *Arch Gen Psychiatry* 51:687–697.
- Bhatnagar S, Huber R, Nowak N, Trotter P (2002) Lesions of the posterior paraventricular thalamus block habituation of hypothalamic-pituitary-adrenal responses to repeated restraint. *J Neuroendocrinol* 14:403–410.
- Blakely RD, Ramamoorthy S, Schroeter S, Qian Y, Apparsundaram S, Galli A, DeFelice LJ (1998) Regulated phosphorylation and trafficking of antidepressant-sensitive serotonin transporter proteins. *Biol Psychiatry* 44:169–178.
- Cannon DM, Ichise M, Rollis D, Klaver JM, Gandhi SK, Charney DS, Manji HK, Drevets WC (2007) Elevated serotonin transporter binding in major depressive disorder assessed using positron emission tomography and C-11 DASB: comparison with bipolar disorder. *Biological Psychiatry* 62:870–877.
- Cannon DM, Klaver JM, Klug SA, Carlson PJ, Luckenbaugh DA, Ichise M, Drevets WC (2013) Gender-specific abnormalities in the serotonin transporter system in panic disorder. *Int J Neuropsychopharmacol* 16:733–743.
- Cools R, Roberts AC, Robbins TW (2008) Serotonergic regulation of emotional and behavioural control processes. *Trends Cogn Sci* 12:31–40.
- Costa PT Jr, McCrae RR (1992a) Revised NEO personality inventory (NEO-PI-R) and NEO five factor inventory (NEO-FFI) professional manual. Odessa, FL: Psychological Assessment Resources.
- Costa PT Jr, McCrae RR (1992b) NEO PI-R professional manual. Odessa, FL: Psychological Assessment Resources, Inc.
- Emond P, Vercouillie J, Innis R, Chalon S, Mavel S, Frangin Y, Hall-din C, Besnard JC, Guilloteau D (2002) Substituted diphenyl

- sulfides as selective serotonin transporter ligands: synthesis and in vitro evaluation. *J Med Chem* 45:1253–1258.
- Erritzoe D, Frokjaer VG, Haahr MT, Kalbitzer J, Svarer C, Holst KK, Hansen DL, Jernigan TL, Lehel S, Knudsen GM (2010) Cerebral serotonin transporter binding is inversely related to body mass index. *Neuroimage* 52:284–289.
- Fisher PM, Haahr ME, Jensen CG, Frokjaer VG, Siebner HR, Knudsen GM (2015) Fluctuations in [(11)C]SB207145 PET binding associated with change in threat-related amygdala reactivity in humans. *Neuropsychopharmacology* 40:1510–1518.
- Frey BN, Skelin I, Sakai Y, Nishikawa M, Diksic M (2010) Gender differences in alpha-[(11)C]MTrp brain trapping, an index of serotonin synthesis, in medication-free individuals with major depressive disorder: a positron emission tomography study. *Psychiatry Res* 183:157–166.
- Frokjaer VG, Mortensen EL, Nielsen FA, Haugbol S, Pinborg LH, Adams KH, Svarer C, Hasselbalch SG, Holm S, Paulson OB, Knudsen GM (2008) Frontolimbic serotonin 2A receptor binding in healthy subjects is associated with personality risk factors for affective disorder. *Biological Psychiatry* 63:569–576.
- Frokjaer VG, Pinborg A, Holst KK, Overgaard A, Henningson S, Heede M, Larsen EC, Jensen PS, Agn M, Nielsen AP, Stenbæk DS, da Cunha-Bang S, Lehel S, Siebner HR, Mikkelsen JD, Svarer C, Knudsen GM (2015) Role of serotonin transporter changes in depressive responses to sex-steroid hormone manipulation: a positron emission tomography study. *Biol Psychiatry* 78:534–543.
- Gressier F, Calati R, Serretti A (2016) 5-HTTLPR and gender differences in affective disorders: a systematic review. *J Affect Disord* 190:193–207.
- Heinz A, Higley JD, Gorey JG, Saunders RC, Jones DW, Hommer D, Zajicek K, Suomi SJ, Lesch KP, Weinberger DR, Linnoila M (1998) In vivo association between alcohol intoxication, aggression, and serotonin transporter availability in nonhuman primates. *Am J Psychiatry* 155:1023–1028.
- Heinz A, Jones DW, Bissette G, Hommer D, Ragan P, Knable M, Wellek S, Linnoila M, Weinberger DR (2002) Relationship between cortisol and serotonin metabolites and transporters in alcoholism [correction of alcoholism]. *Pharmacopsychiatry* 35:127–134.
- Hettema JM, Neale MC, Myers JM, Prescott CA, Kendler KS (2006) A population-based twin study of the relationship between neuroticism and internalizing disorders. *Am J Psychiatry* 163:857–864.
- Hirvonen J, Tuominen L, Någren K, Hietala J (2015) Neuroticism and serotonin 5-HT_{1A} receptors in healthy subjects. *Psychiatry Res* 234:1–6.
- Houle S, Ginovart N, Hussey D, Meyer JH, Wilson AA (2000) Imaging the serotonin transporter with positron emission tomography: initial human studies with [(11)C]DAPP and [(11)C]DASB. *Eur J Nuc Med* 27:1719–1722.
- Ichise M, Liow JS, Lu JQ, Takano A, Model K, Toyama H, Suhara T, Suzuki K, Innis RB, Carson RE (2003) Linearized reference tissue parametric imaging methods: application to [(11)C]DASB positron emission tomography studies of the serotonin transporter in human brain. *J Cereb Blood Flow Metab* 23:1096–1112.
- Innis RB, et al. (2007) Consensus nomenclature for in vivo imaging of reversibly binding radioligands. *J Cereb Blood Flow Metab* 27:1533–1539.
- Jovanovic H, Karlsson P, Cerin A, Halldin C, Nordström AL (2009) 5-HT_{1A} receptor and 5-HTT binding during the menstrual cycle in healthy women examined with [(11)C]WAY100635 and [(11)C]MADAM PET. *Psychiatry Res* 172:31–37.
- Kalbitzer J, Frokjaer VG, Erritzoe D, Svarer C, Cumming P, Nielsen FA, Hashemi SH, Baaré WF, Madsen J, Hasselbalch SG, Kringelbach ML, Mortensen EL, Knudsen GM (2009) The personality trait openness is related to cerebral 5-HTT levels. *Neuroimage* 45:280–285.
- Kalbitzer J, Erritzoe D, Holst KK, Nielsen FA, Marner L, Lehel S, Arentzen T, Jernigan TL, Knudsen GM (2010) Seasonal changes in brain serotonin transporter binding in short serotonin transporter linked polymorphic region-allele carriers but not in long-allele homozygotes. *Biol Psychiatry* 67:1033–1039.
- Kaufman J, Sullivan GM, Yang J, Ogden RT, Miller JM, Oquendo MA, Mann JJ, Parsey RV, DeLorenzo C (2015) Quantification of the serotonin 1A receptor using PET: identification of a potential biomarker of major depression in males. *Neuropsychopharmacology* 40:1692–1699.
- Kendler KS, Gardner CO (2014) Sex differences in the pathways to major depression: a study of opposite-sex twin pairs. *Am J Psychiatry* 171:426–435.
- Kendler KS, Gatz M, Gardner CO, Pedersen NL (2006) Personality and major depression: a Swedish longitudinal, population-based twin study. *Arch Gen Psychiatry* 63:1113–1120.
- Kim JS, Ichise M, Sangare J, Innis RB (2006) PET imaging of serotonin transporters with [(11)C]DASB: test-retest reproducibility using a multilinear reference tissue parametric imaging method. *J Nucl Med* 47:208–214.
- Klaver JM, Drevets WC, Cannon DM (2007) Serotonin transporter binding and personality in healthy subjects assessed using PET and C-11 DASB. *Biological Psychiatry* 61:217S–217S.
- Kranz GS, Wadsak W, Kaufmann U, Savli M, Baldinger P, Gryglewski G, Haeusler D, Spies M, Mitterhauser M, Kasper S, Lanzenberger R (2015) High-dose testosterone treatment increases serotonin transporter binding in transgender people. *Biol Psychiatry* 78:525–533.
- Kugaya A, Epperson CN, Zoghbi S, van Dyck CH, Hou Y, Fujita M, Staley JK, Garg PK, Seibyl JP, Innis RB (2003) Increase in prefrontal cortex serotonin 2A receptors following estrogen treatment in postmenopausal women. *Am J Psychiatry* 160:1522–1524.
- Lammertsma AA, Hume SP (1996) Simplified reference tissue model for PET receptor studies. *Neuroimage* 4:153–158.
- Larsen WA, McCleary SJ (1972) The use of partial residual plots in regression analysis. *Technometrics* 14:781–790.
- Lesch KP, Bengel D, Heils A, Sabol SZ, Greenberg BD, Petri S, Benjamin J, Müller CR, Hamer DH, Murphy DL (1996) Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. *Science* 274:1527–1531.
- Lindeman S, Hämäläinen J, Isometsä E, Kaprio J, Poikolainen K, Heikkinen M, Aro H (2000) The 12-month prevalence and risk factors for major depressive episode in Finland: representative sample of 5993 adults. *Acta Psychiatr Scand* 102:178–184.
- Lundberg J, Halldin C, Farde L (2006) Measurement of serotonin transporter binding with PET and [(11)C]MADAM: a test-retest reproducibility study. *Synapse* 60:256–263.
- Lundberg J, Odano I, Olsson H, Halldin C, Farde L (2005) Quantification of 11C-MADAM binding to the serotonin transporter in the human brain. *J Nuc Med* 46:1505–1515.
- Marcus SM, Kerber KB, Rush AJ, Wisniewski SR, Nierenberg A, Balasubramani GK, Ritz L, Kornstein S, Young EA, Trivedi MH (2008) Sex differences in depression symptoms in treatment-seeking adults: confirmatory analyses from the Sequenced Treatment Alternatives to Relieve Depression study. *Compr Psychiatry* 49:238–246.

- Maron E, Töru I, Hirvonen J, Tuominen L, Lumme V, Vasar V, Shlik J, Nutt DJ, Helin S, Någren K, Tiihonen J, Hietala J (2011) Gender differences in brain serotonin transporter availability in panic disorder. *J Psychopharmacol* 25:952–959.
- Martin LA, Neighbors HW, Griffith DM (2013) The experience of symptoms of depression in men vs women: analysis of the National Comorbidity Survey Replication. *JAMA Psychiatry* 70:1100–1106.
- Mc Mahon B, Andersen SB, Madsen MK, Hjordt LV, Hageman I, Dam H, Svarer C, da Cunha-Bang S, Baaré W, Madsen J, Hasholt L, Holst K, Frokjaer VG, Knudsen GM (2016) Seasonal difference in brain serotonin transporter binding predicts symptom severity in patients with seasonal affective disorder. *Brain* 139:1605–1613.
- Meyer JH (2007) Imaging the serotonin transporter during major depressive disorder and antidepressant treatment. *J Psychiatry Neurosci* 32:86–102.
- Minelli A, Bonvicini C, Scassellati C, Sartori R, Gennarelli M (2011) The influence of psychiatric screening in healthy populations selection: a new study and meta-analysis of functional 5-HTTLPR and rs25531 polymorphisms and anxiety-related personality traits. *BMC Psychiatry* 11:50.
- Moses EL, Drevets WC, Smith G, Mathis CA, Kalro BN, Butters MA, Leondires MP, Greer PJ, Lopresti B, Loucks TL, Berga SL (2000) Effects of estradiol and progesterone administration on human serotonin 2A receptor binding: a PET study. *Biol Psychiatry* 48:854–860.
- Moses-Kolko EL, Berga SL, Greer PJ, Smith G, Cidis Meltzer C, Drevets WC (2003) Widespread increases of cortical serotonin type 2A receptor availability after hormone therapy in euthymic postmenopausal women. *Fertil Steril* 80:554–559.
- Munafo MR, Freimer NB, Ng W, Ophoff R, Veijola J, Miettunen J, Jarvelin MR, Taanila A, Flint J (2009) 5-HTTLPR genotype and anxiety-related personality traits: a meta-analysis and new data. *Am J Med Genet B Neuropsychiatr Genet* 150B:271–281.
- Nishizawa S, Benkelfat C, Young SN, Leyton M, Mzengeza S, de Montigny C, Blier P, Diksic M (1997) Differences between males and females in rates of serotonin synthesis in human brain. *Proc Natl Acad Sci U S A* 94:5308–5313.
- Parsey RV, Oquendo MA, Simpson NR, Ogden RT, Van Heertum R, Arango V, Mann JJ (2002) Effects of sex, age, and aggressive traits in man on brain serotonin 5-HT1A receptor binding potential measured by PET using [C-11]WAY-100635. *Brain Res* 954:173–182.
- Praschak-Rieder N, Willeit M, Wilson AA, Houle S, Meyer JH (2008) Seasonal variation in human brain serotonin transporter binding. *Arch Gen Psychiatry* 65:1072–1078.
- Price JL, Drevets WC (2010) Neurocircuitry of mood disorders. *Neuropsychopharmacology* 35:192–216.
- Sakai Y, Nishikawa M, Leyton M, Benkelfat C, Young SN, Diksic M (2006) Cortical trapping of alpha-[(11)C]methyl-l-tryptophan, an index of serotonin synthesis, is lower in females than males. *Neuroimage* 33:815–824.
- Savitz JB, Drevets WC (2013) Neuroreceptor imaging in depression. *Neurobiol Dis* 52:49–65.
- Sharp T, Cowen PJ (2011) 5-HT and depression: is the glass half-full? *Curr Opin Pharmacol* 11:45–51.
- Shrestha S, Hirvonen J, Hines CS, Henter ID, Svenningsson P, Pike VW, Innis RB (2012) Serotonin-1A receptors in major depression quantified using PET: controversies, confounds, and recommendations. *Neuroimage* 59:3243–3251.
- Spencer SJ, Fox JC, Day TA (2004) Thalamic paraventricular nucleus lesions facilitate central amygdala neuronal responses to acute psychological stress. *Brain Res* 997:234–237.
- Su L, Cai Y, Xu Y, Dutt A, Shi S, Bramon E (2014) Cerebral metabolism in major depressive disorder: a voxel-based meta-analysis of positron emission tomography studies. *BMC Psychiatry* 14:321.
- Svarer C, Madsen K, Hasselbalch SG, Pinborg LH, Haugbøl S, Frøkjær VG, Holm S, Paulson OB, Knudsen GM (2005) MR-based automatic delineation of volumes of interest in human brain PET images using probability maps. *Neuroimage* 24:969–979.
- Takano A, Arakawa R, Hayashi M, Takahashi H, Ito H, Suhara T (2007) Relationship between neuroticism personality trait and serotonin transporter binding. *Biol Psychiatry* 62:588–592.
- Tuominen L, Nummenmaa L, Keltikangas-Järvinen L, Raitakari O, Hietala J (2014) Mapping neurotransmitter networks with PET: an example on serotonin and opioid systems. *Hum Brain Mapp* 35:1875–1884.
- Tuominen L, Salo J, Hirvonen J, Någren K, Laine P, Melartin T, Isometsä E, Viikari J, Cloninger CR, Raitakari O, Hietala J, Keltikangas-Järvinen L (2012) Temperament, character and serotonin activity in the human brain: a positron emission tomography study based on a general population cohort. *Psychol Med*:1–14.
- Vainik U, Dagher A, Dubé L, Fellows LK (2013) Neurobehavioural correlates of body mass index and eating behaviours in adults: a systematic review. *Neurosci Biobehav Rev* 37:279–299.
- Wade TJ, Cairney J, Pevalin DJ (2002) Emergence of gender differences in depression during adolescence: national panel results from three countries. *J Am Acad Child Adolesc Psychiatry* 41:190–198.
- Willeit M, Praschak-Rieder N (2010) Imaging the effects of genetic polymorphisms on radioligand binding in the living human brain: a review on genetic neuroreceptor imaging of monoaminergic systems in psychiatry. *Neuroimage* 53:878–892.
- Wilson AA, Ginovart N, Hussey D, Meyer J, Houle S (2002) In vitro and in vivo characterisation of [11C]-DASB: a probe for in vivo measurements of the serotonin transporter by positron emission tomography. *Nucl Med Biol* 29:509–515.