

RESEARCH ARTICLE

# No evidence for a role of the serotonin 4 receptor in five-factor personality traits: A positron emission tomography brain study

Dea Siggaard Stenbæk<sup>1,2</sup>\*, Vibeke Høyrup Dam<sup>1,2</sup>, Patrick MacDonald Fisher<sup>1,2</sup>, Nanna Hansen<sup>1</sup>, Liv Vadskjær Hjordt<sup>1,2,3</sup>, Vibe Gedsoe Frokjaer<sup>1,2</sup>

**1** Neurobiology Research Unit, the Neuroscience Centre, Copenhagen University Hospital Rigshospitalet, Copenhagen, Denmark, **2** Center for Integrated Molecular Brain Imaging and Center for Experimental Medicine Neuropharmacology, Copenhagen University Hospital Rigshospitalet, Copenhagen, Denmark, **3** Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark

✉ These authors contributed equally to this work.

\* [dea@nru.dk](mailto:dea@nru.dk)



**OPEN ACCESS**

**Citation:** Stenbæk DS, Dam VH, Fisher PMDonald, Hansen N, Hjordt LV, Frokjaer VG (2017) No evidence for a role of the serotonin 4 receptor in five-factor personality traits: A positron emission tomography brain study. PLoS ONE 12(9): e0184403. <https://doi.org/10.1371/journal.pone.0184403>

**Editor:** Aviv M. Weinstein, Ariel University, ISRAEL

**Received:** May 3, 2017

**Accepted:** August 23, 2017

**Published:** September 7, 2017

**Copyright:** © 2017 Stenbæk et al. This is an open access article distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

**Data Availability Statement:** All relevant data are within the paper and its Supporting Information files.

**Funding:** The database study was supported by The Danish Council for Independent Research (grant number 271-07-0651, <http://ufm.dk/en/research-and-innovation/councils-and-commissions/the-danish-council-for-independent-research>) and The Lundbeck Foundation (grant number R90-A7722, <http://www.lundbeckfonden.com/>). The funders had no role in study design,

## Abstract

Serotonin (5-HT) brain architecture appears to be implicated in normal personality traits as supported by genetic associations and studies using molecular brain imaging. However, so far, no studies have addressed potential contributions to variation in normal personality traits from *in vivo* serotonin 4 receptor (5-HT<sub>4</sub>R) brain availability, which has recently become possible to image with Positron Emission Tomography (PET). This is particularly relevant since availability of 5-HT<sub>4</sub>R has been shown to adapt to synaptic levels of 5-HT and thus offers information about serotonergic tone in the healthy brain. In 69 healthy participants (18 females), the associations between personality traits assessed with the five-factor NEO Personality Inventory-Revised (NEO PI-R) and regional cerebral 5-HT<sub>4</sub>R binding in neocortex, amygdala, hippocampus, and anterior cingulate cortex (ACC) were investigated using linear regression models. The associations between each of the five personality traits and a latent variable construct of global 5-HT<sub>4</sub>R levels were also evaluated using latent variable structural equation models. We found no significant associations between the five NEO personality traits and regional 5-HT<sub>4</sub>R binding (all *p*-values > .17) or the latent construct of global 5-HT<sub>4</sub>R levels (all *p*-values > .37). Our findings indicate that NEO personality traits and 5-HT<sub>4</sub>R are not related in healthy participants. Under the assumption that global 5-HT<sub>4</sub>R levels index 5-HT tone, our data also suggest that 5-HT tone per se is not directly implicated in normal personality traits.

## Introduction

Serotonin (5-HT) is a neurotransmitter involved in multiple normal human behaviors, including sleep, appetite, stress regulation, memory, and emotion processing [1] and in a wide range of neuropsychiatric disorders [2–4]. In healthy humans, 5-HT signaling is proposed to play an important role in personality function although the mechanisms involved are far from clearly

data collection and analysis, decision to publish, or preparation of the manuscript.

**Competing interests:** VF has received honorarium as speaker for H. Lundbeck A/S. All other authors have declared that no competing interests exist. This does not alter our adherence to PLOS ONE policies on sharing data and materials.

understood [5, 6]. Some support for an association between the 5-HT system and personality traits is provided by studies of gene variants of the serotonin transporter (5-HTT) [7, 8] and the rate limiting enzyme in serotonin synthesis (TPH2) [9], however, not all genetic studies have found evidence of an association [10–12]. The five-factor model of personality comprises the personality traits Neuroticism (N), Extraversion (E), Openness (O), Agreeableness (A), and Conscientiousness (C). This model has gained wide acceptance due to converging evidence of its stability, heritability, consensual validation, cross-cultural invariance, and predictive utility [13]. Importantly, scores on certain five-factor traits, e.g., high trait N and low trait C, have been associated with risk for psychopathology [14–16], which may be related to dysfunction of the 5-HT system [17]. Thus, mapping serotonergic underpinnings of personality traits in healthy humans may provide critical insights into the molecular basis of vulnerability for psychopathology.

*In vivo* Positron Emission Tomography (PET) techniques have been used to investigate brain 5-HT markers in relation to normal personality traits including the 5-HT<sub>1A</sub> [18–22] and 5-HT<sub>2A</sub> [23–27] receptors as well as the 5-HT transporter (5-HTT) [28–32]. However, methodological issues such as small sample sizes and the use of different psychometric tools to assess personality traits make any overall conclusions difficult. Recently, it has become possible to image the postsynaptic 5-HT<sub>4</sub> receptor (5-HT<sub>4</sub>R) in humans using the PET radioligand [<sup>11</sup>C]SB207145 [33]. From animal studies there is evidence that 5-HT<sub>4</sub>R binding potential (BP<sub>ND</sub>) is inversely related to 5-HT tonus [34, 35], which has been supported by subsequent genetic [36] and pharmacological intervention [37] studies in humans. Furthermore, we have previously shown that in healthy participants, 5-HT<sub>4</sub>R BP<sub>ND</sub> is associated with episodic memory [38, 39], familial risk for depression [40], threat-related reactivity of amygdala [41], which is a key brain structure underlying personality trait N [42], as well as trait aggression in healthy males [43]. Together these findings suggest that the 5-HT<sub>4</sub>R may be linked to stable personality characteristics which warrants further investigation.

Thus, we here for the first time evaluate the association between 5-HT<sub>4</sub>R BP<sub>ND</sub> as imaged with [<sup>11</sup>C]SB207145 PET and five-factor personality traits measured with the NEO Personality Inventory-Revised (NEO PI-R) in healthy participants and hypothesize to detect an association.

## Materials and methods

### Participants

Data from 69 healthy participants (18 females) were available for the study from a large multi-modality database established by the Lundbeck Foundation Center for Integrated Molecular Brain Imaging (Cimbi) [44]. Exclusion criteria for the study were history of significant medical disorders, current or previous psychiatric disorders, head trauma, drug and alcohol abuse, current or former use of psychoactive drugs, regular use of medication, non-fluency in Danish, severe somatic illness, pregnancy or breastfeeding. Somatic and psychiatric screening was performed by a trained clinician and included a neurological examination to exclude anomalies. On scan days, participants were asked about use of regular and p.r.n. medication and in some cases (depending on the study design) a urine test was conducted to screen for drug use. Educational scores were rated on a 5-point Likert scale from 1 (no vocational degree) to 5 (> 4 years of academic education including a prior high school degree). Genotype information was available for all but three participants and included BDNF val66met and 5-HTTLPR polymorphisms known to predict 5-HT<sub>4</sub>R [36]. All studies contributing to this work complied with the Helsinki Declaration. Written informed consent was obtained from all participants and the studies were registered and approved by the Copenhagen municipality and the Capital Region

Ethics Committee (KF01-2006-20, KF0-274821, H-1-2010-085, and KF-2007-0028.). The data in the current study was collected over a period of 7 years (March 2007 to April 2013) and subsets of the included participants have been part of earlier publications on the 5-HT<sub>4</sub>R [33, 36–41, 43, 45, 46].

## Measures

**The NEO Personality Inventory-Revised.** The Danish version of the NEO PI-R was used to assess personality; this version has previously been normed in a sample of 600 individuals [47]. The NEO PI-R is a self-report questionnaire comprising 240 items which measures five traits of personality: N, E, O, A, and C, where each trait consists of six facets [13]. The participants rated each item on a 5-point Likert scale from 0 (strongly disagree) to 4 (strongly agree). The scores of the items loading on each personality trait were summed to a total raw score, which was used in the analyses. Internal consistency (measured with Cronbach's alpha,  $\alpha$ ) for the NEO PI-R traits was high: N,  $\alpha = .94$ ; E,  $\alpha = .90$ ; O,  $\alpha = .87$ ; A,  $\alpha = .90$ ; and C,  $\alpha = .92$ .

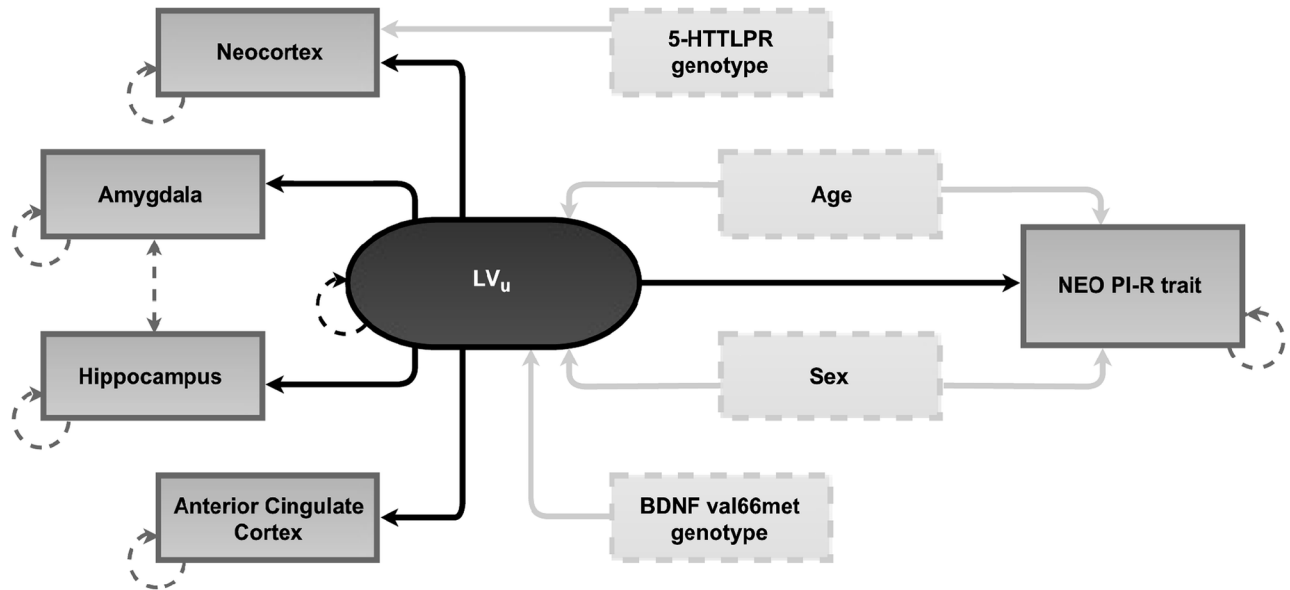
**PET and magnetic resonance imaging (MRI).** The synthesis of [<sup>11</sup>C]SB207145 and quantification of 5-HT<sub>4</sub>R brain BP<sub>ND</sub> was performed according to previously described procedures [33]. Radiochemical purity of the [<sup>11</sup>C]SB207145 tracer ranged from 95–100%. Two different scanners were used for the PET scans: 1) A High Resolution Research Tomograph (HRRT) with an estimated in-plane resolution of 2 mm [48], and 2) an eighteen-ring GE-Advance scanner with an estimated in-plane resolution of 6 mm (GE, Milwaukee, WI). Immediately following the intravenous bolus injection of [<sup>11</sup>C]SB207145, a 120-min dynamic 3D PET scan (6x5s, 10x15s, 4x30s, 5x120s, 5x300s, and 8x600s) was conducted. The acquired PET data was reconstructed as described by Fisher *et al.* [36].

MRI was obtained using a 3T Siemens Magnetom Trio scanner (Erlangen, Germany). 2D T2-weighted and high-resolution 3D T1-weighted sequences (matrix 256 x 256; 1x1x1mm voxels) were acquired and corrected for non-uniformity and spatial distortions. Using SPM5 (Wellcome Department of Cognitive Neurology, London, UK), T1-weighted images were segmented into gray matter, white matter, and cerebrospinal fluid. Each voxel was assigned to the tissue class with the highest probability and this segmentation was applied afterwards for delineation of the region of interest. The T2-weighted images were used for brain masking.

Pvelab was used to automatically outline regions from the structural MRI scan and subsequently determine time-activity curves within each region [49]. The non-displaceable BP<sub>ND</sub> of [<sup>11</sup>C]SB207145 was modeled with the simplified reference tissue model using PMOD (PMOD Technologies, Zurich, Switzerland) employing cerebellum as a reference region [33], defined as:  $BP_{ND} = f_{ND} * B_{avail} * (1/K_D)$ , where  $f_{ND}$  is the free fraction of ligand in the nondisplaceable tissue compartment,  $K_D$  is the dissociation constant, and  $B_{avail}$  is the concentration of receptors available for binding [50]. Four regions were included in our model: Neocortex, amygdala, hippocampus, and anterior cingulate cortex (ACC), as these regions are critically involved in the brain's emotional circuitry [51].

**Genotyping.** Genotyping was performed as described previously for the BDNF val66met [36] and 5-HTTLPR polymorphisms [42]. Genomic DNA was extracted from blood using QIAamp DNA Blood Mini Kit (Qiagen, Valencia, CA). DNA quality and concentration were measured using a UV-Vis spectrophotometer (NanoDrop2000, Thermo Scientific). The participants were subsequently grouped in a bivariate fashion into val-val vs. met carriers for the BDNF val66met and LL vs. S-carriers for the 5-HTTLPR polymorphism.

**Statistics.** The associations between the NEO personality traits and natural log-transformed regional 5-HT<sub>4</sub>R BP<sub>ND</sub> in neocortex, amygdala, hippocampus, and ACC were analyzed using general linear models. Based on previous observations, the models were corrected for



**Fig 1. Schematic overview of the latent variable model for the association between cerebral serotonin 4 receptor (5-HT<sub>4</sub>R) binding potential (BP<sub>ND</sub>) and NEO personality traits.** A separate model was determined for each of the five NEO personality traits: Neuroticism, Extroversion, Openness, Agreeableness and Conscientiousness. The circle represents the latent 5-HT<sub>4</sub>R variable (LV<sub>u</sub>). Light gray boxes indicate observed predictors and the dark gray boxes, predicted by the latent variable, represent observed log-transformed regional 5-HT<sub>4</sub>R BP<sub>ND</sub> and NEO personality trait. Age, gender, and BDNF val66met genotype all map directly onto the latent variable (LV<sub>u</sub>). Age and sex also predict the personality trait and 5-HTTLPR genotype predicts neocortex regional BP<sub>ND</sub> only. Not shown in the model are two observed predictors 1) scanner type (GE-Advance vs. HRRT PET scanner) and 2) weight adjusted injected mass, which map directly onto each regional BP<sub>ND</sub>. The hatched line between amygdala and hippocampus reflects additional shared correlation. Circular hatched lines denote parameters estimated with error.

<https://doi.org/10.1371/journal.pone.0184403.g001>

age, sex [45, 47], 5-HTTLPR and BDNF val66met polymorphisms, weight adjusted injected mass, and PET scanner type [36].

In line with our previous findings [36], we observed high intercorrelation between 5-HT<sub>4</sub>R BP<sub>ND</sub> across the regions of interest (*r*-values ranged from .52 to .95, all *p*-values < .001). To best capture a proxy for this global 5-HT<sub>4</sub>R signal and evaluate its associations with each of the five personality traits, we therefore performed a series of secondary latent variable structural equation model analyses (Fig 1).

Latent variable models can be considered an extension of linear mixed models for the analysis of multiple repeated measurements, which in this case allows us to model the shared correlation between different regional brain BP<sub>ND</sub> as a single variable. A major benefit of using latent variable models, as compared to linear mixed models, is that it allows for some measurements to be more correlated than others (i.e., it can account for the BP<sub>ND</sub> of a brain region to be differentially correlated to other regions). Furthermore, it substantially reduces the penalty associated with multiple comparisons.

In these analyses, a single latent 5-HT<sub>4</sub>R variable (LV<sub>u</sub>) modeled the shared correlation between natural log-transformed regional 5-HT<sub>4</sub>R BP<sub>ND</sub> in neocortex, amygdala, hippocampus, and ACC. BDNF val66met genotype, weight-adjusted injected mass, age, and sex were included as predictors of the latent 5-HT<sub>4</sub>R variable, 5-HTTLPR genotype was modeled as a predictor of neocortex 5-HT<sub>4</sub>R BP<sub>ND</sub>, and PET scanner type was modeled as predictors of 5-HT<sub>4</sub>R BP<sub>ND</sub> for each region. Age and sex were also modeled as predictors for NEO personality traits to account for effects independent of the relation between 5-HT<sub>4</sub>R BP<sub>ND</sub> and NEO PI-R scores [47]. Model fitting and parameters estimates were obtained using the Lava package

in R [52], using neocortex BP<sub>ND</sub> as a reference scale. Additional shared covariance between the amygdala and hippocampus was supported based on Score tests (false-discovery rate  $q < 0.05$ ), which specify whether an additional path would significantly benefit overall model fit, and thus included for all models. Statistical analyses were carried out in SPSS (v22.0) and R (v3.0.2) (R Core Team 2013) with  $p$ -values  $< .05$  considered statistically significant.

## Results

### Descriptive data

Table 1 shows descriptive data for the 69 healthy participants. Age ranged from 20–86 years ( $33 \pm 16$ , mean  $\pm$  SD). The reported scores on the five NEO personality traits did not differ significantly from the Danish norm group [47] on trait N and E ( $p > .22$ ), but our sample showed a significantly higher score on trait O ( $p < .001$ ) and significantly lower scores on trait A ( $p < .01$ ) and C ( $p < .01$ ).

### 5-HT<sub>4</sub>R binding and personality

Consistent with our previous observations [36], the data supported a latent variable model structure, as indicated by a high intercorrelation in 5-HT<sub>4</sub>R BP<sub>ND</sub> between regions (LV<sub>*i*</sub>: all region BP<sub>ND</sub> factor loadings across all models,  $p < 1 \times 10^{-7}$ ). The models for each NEO

**Table 1. Descriptive data.**

Descriptive data (N = 69)	
Age in years	32.9 $\pm$ 15.8 (20–86)
Body Mass Index	23.6 $\pm$ 2.6 (19.1–31.3)
Sex (female/male)	18/51
Tobacco use (smokers/non-smokers)	11/57 <sup>a</sup>
5-HTTLPR genotype (LL/S-carriers)	28/40 <sup>b</sup>
BDNF val66met genotype (val-val/met-carriers)	30/36 <sup>a</sup>
Scanner type (GE-Advanced/HRRT)	20/49
SB specific activity (GBq/mikromol)	126.2 [61.9–211.9]
Neocortex 5-HT <sub>4</sub> BP <sub>ND</sub>	.58 $\pm$ .17 (.24–.88)
Amygdala 5-HT <sub>4</sub> BP <sub>ND</sub>	.85 $\pm$ .18 (.53–1.3)
Hippocampus 5-HT <sub>4</sub> BP <sub>ND</sub>	.96 $\pm$ .18 (.48–1.4)
Anterior Cingulate Cortex 5-HT <sub>4</sub> BP <sub>ND</sub>	.70 $\pm$ .18 (.29–1.1)
Neuroticism	74.0 $\pm$ 21.5 (34–139)
Extraversion	116.2 $\pm$ 18.9 (53–148)
Openness	115.6 $\pm$ 16.5 (73–152)
Agreeableness	119.1 $\pm$ 17.0 (47–152)
Conscientiousness	113.3 $\pm$ 20.0 (72–150)

Descriptive data for the 69 participants with means  $\pm$  standard deviation and range in brackets, ratios, or median and interquartile ranges in square brackets. The table shows age, body mass index, sex, tobacco use, 5-HTTLPR LL- and S-carrier ratio, BDNF val66met val/val and met carrier ratio. Scanner type (GE-Advanced vs. HRRT PET scanner), SB specific activity and 5-HT<sub>4</sub> receptor binding potential (BP<sub>ND</sub>) for neocortex, amygdala, hippocampus, and anterior cingulate cortex are also shown as well as raw scores of the five personality traits: Neuroticism, Extraversion, Openness, Agreeableness, and Conscientiousness measured with the Danish version of the Revised NEO Personality Inventory.

<sup>a</sup> Data is missing for one participant.

<sup>b</sup> Data is missing for three participants.

<https://doi.org/10.1371/journal.pone.0184403.t001>

**Table 2. NEO personality and regional 5-HT<sub>4</sub>R binding potential.**

	Neocortex				Amygdala				Hippocampus				Anterior Cingulate Cortex			
	$\beta$	$p$	95% CI	$r^2$	$\beta$	$p$	95% CI	$r^2$	$\beta$	$p$	95% CI	$r^2$	$\beta$	$p$	95% CI	$r^2$
Neuroticism	-6.5	.79	[-55.8–42.8]	.07	0.43	.98	[-32.0–31.9]	.06	-9.6	.68	[-55.9–36.7]	.07	-14.1	.52	[-57.6–29.4]	.07
Extraversion	26.3	.22	[-16.3–68.8]	.08	9.5	.49	[-17.8–36.9]	.06	27.8	.17	[-12.1–67.6]	.09	8.8	.64	[-29.2–46.9]	.06
Openness	-12.7	.48	[-48.2–22.8]	.14	0.54	.96	[-22.2–23.3]	.13	3.0	.86	[-30.5–36.5]	.13	-11.8	.46	[-43.2–19.6]	.14
Agreeableness	6.3	.74	[-31.9–44.4]	.09	-1.6	.90	[-25.9–22.7]	.08	21.4	.23	[-14.1–56.8]	.11	18.6	.27	[-14.8–52.0]	.11
Conscientiousness	3.8	.86	[-38.8–46.5]	.18	-2.1	.88	[-29.3–25.1]	.18	10.3	.61	[-29.7–50.3]	.18	12.3	.51	[-25.2–49.9]	.18

Associations between NEO personality traits and regional log-transformed 5-HT<sub>4</sub>R binding potential.  $\beta$ , uncorrected  $p$ -values, 95% confidence interval (CI 95%), and  $r^2$ -values are presented. All models were corrected for age, sex, BDNF val66met and 5-HTTLPR genotype, scanner type (Advance vs. HRRT PET scanner), and weight-adjusted injected mass.

<https://doi.org/10.1371/journal.pone.0184403.t002>

personality trait all showed good model fit (all  $X^2 < 25.2$ ,  $df = 20$ ,  $p > .19$ ). The results of the regional analyses are presented in Table 2.

Neither regional 5-HT<sub>4</sub>R BP<sub>ND</sub> in the neocortex, amygdala, hippocampus or ACC nor the latent 5-HT<sub>4</sub>R level (LV<sub>u</sub>) showed significant associations with any of the five NEO personality traits (all  $p$ -values  $> .17$ ). Neocortex was assigned as the reference region in the latent variable model. Thus, the LV<sub>u</sub> is interpreted in terms of neocortex BP<sub>ND</sub>, i.e., the presented estimates can be read as changes in personality trait score per unit change in neocortex BP<sub>ND</sub>. The results from the latent variable models are presented in Table 3.

### Discussion

This is the first study to investigate the association between *in vivo* 5-HT<sub>4</sub>R BP<sub>ND</sub> and NEO personality traits in humans. Contrary to our expectations, neither regional 5-HT<sub>4</sub>R BP<sub>ND</sub> in the neocortex, amygdala, hippocampus, and ACC nor the latent 5-HT<sub>4</sub>R level were significantly associated with any of the five NEO personality traits in healthy participants.

Although we observed no association between 5-HT<sub>4</sub>R BP<sub>ND</sub> and normal personality traits, *in vivo* PET studies with other brain imaging markers of the 5-HT system have been applied to study this relation. Based on these studies, personality trait N appears to be most consistently linked to features of the 5-HT system: Hirvonen *et al.* [19] reported an inverse association between trait N and global 5-HT<sub>1A</sub> receptor (5-HT<sub>1A</sub>R) BP<sub>ND</sub> in healthy participants ( $n = 34$ ), however, two other studies did not observe this relation (Karlsson *et al.* [20] ( $n = 20$ ), Rabiner *et al.* [21] ( $n = 44/49$ )). Frokjaer *et al.* found that trait N was positively associated with fronto-limbic 5-HT<sub>2A</sub> receptor (5-HT<sub>2A</sub>R) BP<sub>ND</sub> in 83 healthy participants [23] and in 21 healthy

**Table 3. Latent variable model path analyses.**

Path analyses	$\beta$	SE	$p$	95% CI	$r^2$
Neuroticism $\leftarrow$ U	-1.0	17.7	.95	[-35.8–33.8]	$< .001$
Extraversion $\leftarrow$ U	20.5	16.1	.20	[-11.1–52.1]	.05
Openness $\leftarrow$ U	-6.4	13.7	.64	[-33.4–20.5]	.006
Agreeableness $\leftarrow$ U	3.2	11.8	.79	[-19.9–26.3]	.001
Conscientiousness $\leftarrow$ U	-1.5	15.4	.92	[-31.7–28.7]	$< .001$

Model path analysis values for each of the five latent variable models comparing underlying cerebral 5-HT<sub>4</sub>R binding potential to the NEO personality traits.  $\beta$ , standard error (SE),  $p$ -values, 95% confidence interval (95% CI), and  $r^2$ -values are presented for each of the five model runs.  $P$ -values denote the significance of each model path and are uncorrected for multiple comparisons.

<https://doi.org/10.1371/journal.pone.0184403.t003>

participants with a familial risk of mood disorder [24]. Furthermore, Takano *et al.* [30] reported a positive correlation between trait N and 5-HTT BP<sub>ND</sub> in thalamus (n = 31). Together, these findings support a coupling between certain features of the 5-HT system and personality traits associated with vulnerability for psychiatric disorders [17]. Our data intriguingly suggest that these earlier observed associations between trait N and postsynaptic features of the 5-HT system and 5-HTT are uncoupled from an association with 5-HT<sub>4</sub>R, which presumably captures variations in synaptic 5-HT levels.

High levels of trait N have previously been found to correlate with increased levels of aggression [53]. Therefore, the findings that trait N is coupled with certain features of the 5-HT system correspond with the findings from da Cunha-Bang *et al.* [43] who reported a significant positive association between 5-HT<sub>4</sub>R BP<sub>ND</sub> and trait aggression in males in a subset of the participants included in the present study. However, the fact that we did not observe any association between 5-HT<sub>4</sub>R BP<sub>ND</sub> and NEO personality traits suggests that the variance in trait aggression captured by 5-HT<sub>4</sub>R BP<sub>ND</sub> may be different from the variance shared between NEO personality traits and trait aggression. When we tested the overlap between trait aggression and trait N, we found a shared variance of approximately 25% in our population, supporting that the two could very well be differentially associated with features within the 5-HT system.

Other normal personality traits have shown sporadic associations with 5-HT neurotransmission: Gerretsen *et al.* [25] (n = 24) reported a negative association between 5-HT<sub>2A</sub>R BP<sub>ND</sub> and trait Reward Dependence measured with the Temperament and Character Inventory (TCI) [54], while Soloff *et al.* [27] found a positive correlation between 5-HT<sub>2A</sub>R BP<sub>ND</sub> and trait Persistence measured with TCI (n = 21). Lastly, Kalbitzer *et al.* [28] found that 5-HTT BP<sub>ND</sub> was negatively related to trait O (n = 50), while Tuominen *et al.* [32] observed a positive association between 5-HTT BP<sub>ND</sub> and trait Self-directedness measured with TCI (n = 22). Notably, only Kalbitzer *et al.* [28] used NEO PI-R to index normal personality traits while the remaining studies used TCI which makes any direct comparisons to our results difficult. In addition, only a few studies used correction for multiple comparisons or compared the personality trait scores of the included participants to established norm data. This may potentially have weakened the strength and generalizability of the reported findings. In reviewing these results, it appears that no clear trend exists with regard to associations between other (i.e., non-trait N) personality traits and 5-HT neurotransmission. Note, only studies with sample sizes  $\geq 20$  healthy participants are included above. A full review of the existing literature can be found in supporting information (S1 Table).

Given that 5-HT<sub>4</sub>R BP<sub>ND</sub> has previously been linked to fluctuations in 5-HT tone within the brain [37], our negative finding implies that normal personality traits are not directly related to synaptic 5-HT in healthy participants. Intriguingly, other features of the 5-HT brain architecture which are strongly genetically determined, such as the 5-HT<sub>2A</sub>R [55], or partly represent serotonergic wiring, namely 5-HT neurons and axons such as the 5-HTT [56], do appear to correlate with normal personality traits in healthy participants, especially trait N, as reviewed above. We speculate that the wiring of the 5-HT system predominantly established early in brain development [57, 58], rather than dynamic variations in 5-HT tone, is related to long-term stable personality constructs. This also aligns with the notion that NEO personality traits represent stable psychological constructs, which are established early in brain- and psychological development and maturation [13].

## Methodological considerations

While this is the second largest PET study to examine the association between imaging markers of 5-HT signaling and normal personality traits, some limitations of the study should be

considered. First, given our sample size of 69 participants, we have sufficient statistical power ( $\beta = .8$ ) to detect medium to large effect sizes of  $r^2 \geq .09$ . Thus, while it is unlikely that we have overlooked any medium to large effects, we cannot exclude the possibility that potential small effects were not detected. However, we argue that the practical relevance of such small effects may be negligible since a change of one standard deviation in  $BP_{ND}$  (14.8%) in our sample corresponds to a change in a given NEO personality trait smaller than the population-based SEM reported for this trait, i.e., the amount of inaccuracy or error inherent in the measured trait [47]. Second, we were not able to address the potential moderating role of sex for the investigated association between 5-HT<sub>4</sub>R  $BP_{ND}$  and NEO personality traits due to the limited number of women included. Given that da Cunha-Bang *et al.* [43] found a significant effect of sex for the association between trait aggression and 5-HT<sub>4</sub>R  $BP_{ND}$ , combined with earlier reported sex differences in 5-HT<sub>4</sub>R  $BP_{ND}$  [45], this will be relevant to consider in future studies. Third, participants with current and previous psychiatric disorders were excluded from our study population and we may therefore have underestimated a potential link between 5-HT<sub>4</sub>R  $BP_{ND}$  and NEO personality traits in more vulnerable populations, e.g., participants recovered from depression and/or with a family history of mood disorders. Fourth, self-report biases are inherent methodological issues with questionnaire data, e.g., censorship, social desirability biases, or systematic manipulation of answers on items [59]. However, studies of the correlation between self-report scores and ratings by spouse on the NEO PI-R supports the reliability of the traits measured [13].

## Conclusion

We observed no association between normal personality traits and 5-HT<sub>4</sub>R  $BP_{ND}$  in a large group of healthy participants. Under the assumption that global 5-HT<sub>4</sub>R levels index 5-HT tone, our data suggest that 5-HT tone per se is not directly implicated in stable personality traits in the adult healthy brain.

## Supporting information

### S1 Table. Review of studies investigating serotonin markers and normal personality.

Review of PET studies investigating the association between serotonin (5-HT) markers and normal personality in healthy participants. *Personality*: EPQ = Eysenck Personality Questionnaire, KSP = Karolinska Scales of Personality, NEO PI-R = Revised NEO Personality Inventory, TCI = Temperament and Character Inventory, TPQ = Tridimensional Personality Questionnaire. *Brain*: 5-HT = serotonin, 5-HT<sub>1A</sub> = serotonin 1A receptor, 5-HT<sub>2A</sub> = Serotonin 2A receptor, 5-HTT = serotonin transporter, ACC = anterior cingulate cortex,  $BP_{ND}$  = Binding potential, DLPC = dorsolateral prefrontal cortex, FC = frontal cortex, LPC = left parietal cortex, lt.MFC = left medial frontal cortex, OC = occipital cortex, OFC = orbito frontal cortex PC = parietal cortex, RN = raphe nuclei. (DOCX)

## Acknowledgments

We would like to thank GM. Knudsen for sponsoring the study and B. Dall, G. Thomsen, S. Larsen, A. Dyssegaard, and L. Freyr for their assistance with the data collection. We would also like to acknowledge and thank The John and Birthe Meyer Foundation for the donation of the Cyclotron and PET scanner.



## Author Contributions

**Conceptualization:** Dea Siggaard Stenbæk, Vibeke Høyrup Dam, Vibe Gedsoe Frokjaer.

**Data curation:** Vibeke Høyrup Dam.

**Formal analysis:** Dea Siggaard Stenbæk, Vibeke Høyrup Dam, Patrick MacDonald Fisher, Vibe Gedsoe Frokjaer.

**Investigation:** Dea Siggaard Stenbæk, Vibeke Høyrup Dam, Nanna Hansen, Liv Vadskjær Hjordt.

**Methodology:** Dea Siggaard Stenbæk, Vibeke Høyrup Dam, Patrick MacDonald Fisher, Vibe Gedsoe Frokjaer.

**Software:** Patrick MacDonald Fisher.

**Supervision:** Vibe Gedsoe Frokjaer.

**Visualization:** Vibeke Høyrup Dam.

**Writing – original draft:** Dea Siggaard Stenbæk, Vibeke Høyrup Dam, Nanna Hansen.

**Writing – review & editing:** Dea Siggaard Stenbæk, Vibeke Høyrup Dam, Patrick MacDonald Fisher, Liv Vadskjær Hjordt, Vibe Gedsoe Frokjaer.

## References

1. Müller PC, Jacobs BL. Handbook of the Behavioral Neurobiology of Serotonin. London, UK: Academic Press; 2010.
2. Geldenhuys WI, Van der Schyf CI. Role of Serotonin in Alzheimer's Disease. *CNS Drugs*. 2011; 25(9): 765–81. <https://doi.org/10.2165/11590190-000000000-00000> PMID: 21870888.
3. Selvaraj S, Arnone D, Cappai A, Howes O. Alterations in the serotonin system in schizophrenia: A systematic review and meta-analysis of postmortem and molecular imaging studies. *Neuroscience & Biobehavioral Reviews*. 2014 9//; 45(0):233–45.
4. Krishnan V, Nestler E. The molecular neurobiology of depression. *Nature*. 2008; 455(7215):894–902. <https://doi.org/10.1038/nature07455> PMID: 18923511
5. Carver CS, Miller CJ. Relations of serotonin function to personality: Current views and a key methodological issue. *Psychiatry research*. 2006; 144(1):1–15. <https://doi.org/10.1016/j.psychres.2006.03.013> PMID: 16914207
6. Deyoung CG. Personality Neuroscience and the Biology of Traits. Oxford, UK 2010. p. 1165–80.
7. Munafò MR, Freimer NB, Ng W, Ophoff R, Veijola J, Miettunen J, et al. 5-HTTLPR genotype and anxiety-related personality traits: A meta-analysis and new data. *American Journal of Medical Genetics Part B: Neuropsychiatric Genetics*. 2009; 150(2):271–81.
8. Sen S, Burmeister M, Ghosh D. Meta-analysis of the association between a serotonin transporter promoter polymorphism (5-HTTLPR) and anxiety-related personality traits. *American Journal of Medical Genetics Part B: Neuropsychiatric Genetics*. 2004; 127(1):85–9.
9. Lehto K, Vaht M, Maestu J, Veidebaum T, Harro J. Effect of tryptophan hydroxylase-2 gene polymorphism G-703 T on personality in a population representative sample. *Progress in Neuropsychopharmacology & Biological Psychiatry*. 2015; 57:31.
10. Lo M-T, Hinds DA, Tung JY, Franz C, Fan C-C, Wang Y, et al. Genome-wide analyses for personality traits identify six genomic loci and show correlations with psychiatric disorders. *Nat Genet*. 2017 01// print; 49(1):152–6. <https://doi.org/10.1038/ng.3736> PMID: 27918536
11. Middeldorp C, Geus E, Beem A, Lakenberg N, Hottenga J-J, Slagboom P, et al. Family Based Association Analyses between the Serotonin Transporter Gene Polymorphism (5-HTTLPR) and Neuroticism, Anxiety and Depression. *Behavior Genetics*. 2007; 37(2):294–301. <https://doi.org/10.1007/s10519-006-9139-7> PMID: 17216342
12. Plieger T, Montag C, Felten A, Reuter M. The serotonin transporter polymorphism (5-HTTLPR) and personality: response style as a new endophenotype for anxiety. *Int J Neuropsychopharmacol*. 2014; 17(6):851–8. <https://doi.org/10.1017/S1461145713001776> PMID: 24438559

13. Costa JPT, McCrae RR. *Personality in Adulthood, A Five-Factor Theory Perspective*. Hoboken: Taylor & Francis; 2005.
14. Kendler K, Myers J. The genetic and environmental relationship between major depression and the five-factor model of personality. *Psychological medicine*. 2010; 40(05):801–6.
15. Kotov R, Gamez W, Schmidt F, Watson D. Linking "Big" Personality Traits to Anxiety, Depressive, and Substance Use Disorders: A Meta-Analysis. *Psychological Bulletin*. 2010; 136(5):768–821. <https://doi.org/10.1037/a0020327> PMID: 20804236
16. Malouff J, Thorsteinsson E, Schutte N. The Relationship Between the Five-Factor Model of Personality and Symptoms of Clinical Disorders: A Meta-Analysis. *Journal of Psychopathology and Behavioral Assessment*. 2005; 27(2):101–14.
17. Jans LAW, Riedel WJ, Markus CR, Blokland A. Serotonergic vulnerability and depression: assumptions, experimental evidence and implications. *Molecular Psychiatry*. 2006; 12(6):522. <https://doi.org/10.1038/sj.mp.4001920> PMID: 17160067
18. Borg J, Andrée B, Soderstrom H, Farde L. The serotonin system and spiritual experiences. *American Journal of Psychiatry*. 2003; 160(11):1965–9. <https://doi.org/10.1176/appi.ajp.160.11.1965> PMID: 14594742
19. Hirvonen J, Tuominen L, Nagren K, Hietala J. Neuroticism and serotonin 5-HT1A receptors in healthy subjects. *Psychiatry research*. 2015 Oct 30; 234(1):1–6. <https://doi.org/10.1016/j.psychresns.2015.04.007> PMID: 26337006. Epub 2015/09/05. eng.
20. Karlsson H, Karlsson J, Hirvonen JK, Hietala J, Hirvonen J, Salminen J. No association between serotonin 5-HT 1A receptors and spirituality among patients with major depressive disorders or healthy volunteers. *Molecular Psychiatry*. 2011; 16(3):282–5. <https://doi.org/10.1038/mp.2009.126> PMID: 19935737
21. Rabiner EA, Messa C, Sargent PA, Husted-Kjaer K, Montgomery A, Lawrence AD, et al. A database of [11 C]WAY-100635 binding to 5-HT 1A receptors in normal male volunteers: Normative data and relationship to methodological, demographic, physiological, and behavioral variables. *NeuroImage*. 2002; 15(3):620–32. <https://doi.org/10.1006/nimg.2001.0984> PMID: 11848705
22. Tauscher J, Bagby RM, Javanmard M, Christensen BK, Kasper S, Kapur S. Inverse relationship between serotonin 5-HT 1A receptor binding and anxiety: A [11 C]WAY-100635 PET investigation in healthy volunteers. *American Journal of Psychiatry*. 2001; 158(8):1326–8. <https://doi.org/10.1176/appi.ajp.158.8.1326> PMID: 11481173
23. Frokjaer VG, Mortensen EL, Nielsen FÅ, Haugbol S, Pinborg LH, Adams KH, et al. Frontolimbic serotonin 2A receptor binding in healthy subjects is associated with personality risk factors for affective disorder. *Biological psychiatry*. 2008; 63(6):569–76. <https://doi.org/10.1016/j.biopsych.2007.07.009> PMID: 17884017
24. Frokjaer V, Vinberg M, Erritzoe D, Baaré W, Holst K, Mortensen E, et al. Familial Risk for Mood Disorder and the Personality Risk Factor, Neuroticism, Interact in Their Association with Frontolimbic Serotonin 2A Receptor Binding. *Neuropsychopharmacology*. 2010; 35(5):1129–37. <https://doi.org/10.1038/npp.2009.218> PMID: 20043006
25. Gerretsen P, Graff-Guerrero A, Menon M, Pollock B, Kapur S, Vasdev N, et al. Is desire for social relationships mediated by the serotonergic system in the prefrontal cortex? An 18 Fsetoperone PET study. *Social Neuroscience*. 2010; 5(4):375–83. <https://doi.org/10.1080/17470911003589309> PMID: 20198536
26. Moresco F, Dieci M, Vita A, Messa C, Gobbo C, Galli L, et al. In vivo serotonin 5HT 2A receptor binding and personality traits in healthy subjects: A positron emission tomography study. *Neuroimage*. 2002; 17(3):1470–8. PMID: 12414286
27. Soloff PH, Price JC, Mason NS, Becker C, Meltzer CC. Gender, personality, and serotonin-2A receptor binding in healthy subjects. *Psychiatry Research—Neuroimaging*. 2010; 181(1):77–84.
28. Kalbitzer J, Frokjaer VG, Erritzoe D, Svarer C, Cumming P, Nielsen FÅ, et al. The personality trait openness is related to cerebral 5-HTT levels. *Neuroimage*. 2009; 45(2):280–5. <https://doi.org/10.1016/j.neuroimage.2008.12.001> PMID: 19135154
29. Reimold M, Batra A, Knobel A, Smolka M, Zimmer A, Mann K, et al. Anxiety is associated with reduced central serotonin transporter availability in unmedicated patients with unipolar major depression: a [C-11]DASB PET study. *Mol Psychiatr*. 2008; 13(6):606–13.
30. Takano A, Arakawa R, Hayashi M, Takahashi H, Ito H, Suhara T. Relationship Between Neuroticism Personality Trait and Serotonin Transporter Binding. *Biological Psychiatry*. 2007; 62(6):588–92. <https://doi.org/10.1016/j.biopsych.2006.11.007> PMID: 17336939
31. Kim JH, Son YD, Kim JH, Choi EJ, Lee SY, Joo YH, et al. Self-transcendence trait and its relationship with in vivo serotonin transporter availability in brainstem raphe nuclei: An ultra-high resolution PET-MRI study. *Brain Res*. 2015 Dec 10; 1629:63–71. <https://doi.org/10.1016/j.brainres.2015.10.006> PMID: 26459992. Epub 2015/10/16. eng.

32. Tuominen L, Hirvonen J, Laine J, Hietala K, Tuominen P, Hirvonen T, et al. Temperament, character and serotonin activity in the human brain: A positron emission tomography study based on a general population cohort. *Psychological Medicine*. 2013; 43(4):881–94. <https://doi.org/10.1017/S003329171200164X> PMID: 22850434
33. Marner L, Gillings N, Comley RA, Baaré WFC, Rabiner EA, Wilson AA, et al. Kinetic Modeling of 11C-SB207145 Binding to 5-HT<sub>4</sub> Receptors in the Human Brain In Vivo. *Journal of Nuclear Medicine*. 2009 June 1, 2009; 50(6):900–8. <https://doi.org/10.2967/jnumed.108.058552> PMID: 19470850
34. Licht C, Marcussen AB, Wegener G, Overstreet D, Aznar S, Knudsen G. The brain 5-HT<sub>4</sub> receptor binding is down-regulated in the Flinders Sensitive Line depression model and in response to paroxetine administration. *J Neurochem*. 2009; 109(5):1363–74. <https://doi.org/10.1111/j.1471-4159.2009.06050.x> PMID: 19476548
35. Vidal R, Valdizan E, Mostany R, Pazos A, Castro E. Long-term treatment with fluoxetine induces desensitization of 5-HT<sub>4</sub> receptor-dependent signalling and functionality in rat brain. *J Neurochem*. 2009; 110(3):1120–7. <https://doi.org/10.1111/j.1471-4159.2009.06210.x> PMID: 19522734
36. Fisher PM, Holst KK, Adamsen D, Klein AB, Frokjaer VG, Jensen PS, et al. BDNF Val66met and 5-HTTLPR polymorphisms predict a human in vivo marker for brain serotonin levels. *Human Brain Mapping*. 2015; 36(1):313–23. <https://doi.org/10.1002/hbm.22630> PMID: 25220079
37. Haahr ME, Fisher PM, Jensen CG, Frokjaer VG, Mahon BM, Madsen K, et al. Central 5-HT<sub>4</sub> receptor binding as biomarker of serotonergic tonus in humans: a 11CSB207145 PET study. *Molecular psychiatry*. 2014; 19(4):427. <https://doi.org/10.1038/mp.2013.147> PMID: 24189342
38. Haahr ME, Fisher P, Holst K, Madsen K, Jensen CG, Marner L, et al. The 5-HT<sub>4</sub> receptor levels in hippocampus correlates inversely with memory test performance in humans. *Human Brain Mapping*. 2012.
39. Stenbaek DS, Fisher PM, Ozenne B, Andersen E, Hjordt LV, McMahon B, et al. Brain serotonin 4 receptor binding is inversely associated with verbal memory recall. *Brain and behavior*. 2017 Apr; 7(4): e00674. <https://doi.org/10.1002/brb3.674> PMID: 28413715. Epub 2017/04/18. eng.
40. Madsen K, Torstensen E, Holst KK, Haahr ME, Knorr U, Frokjaer VG, et al. Familial risk for major depression is associated with lower striatal 5-HT<sub>4</sub> receptor binding. *Int J Neuropsychopharmacol*. 2015 Jan; 18(1). <https://doi.org/10.1093/ijnp/pyu034> PMID: 25522384. Epub 2014/12/19. eng.
41. Fisher PM, Haahr ME, Jensen CG, Frokjaer VG, Siebner HR, Knudsen GM. Fluctuations in [(1)(1)C] SB207145 PET binding associated with change in threat-related amygdala reactivity in humans. *Neuropsychopharmacology*. 2015 May; 40(6):1510–8. <https://doi.org/10.1038/npp.2014.339> PMID: 25560201. Epub 2015/01/07. eng.
42. Madsen MK, Mc Mahon B, Andersen SB, Siebner HR, Knudsen GM, Fisher PM. Threat-related amygdala functional connectivity is associated with 5-HTTLPR genotype and neuroticism. *Social cognitive and affective neuroscience*. 2016 Jan; 11(1):140–9. <https://doi.org/10.1093/scan/nsv098> PMID: 26245837. Epub 2015/08/08. eng.
43. da Cunha-Bang S, Mc Mahon B, Fisher PM, Jensen PS, Svarer C, Knudsen GM. High trait aggression in men is associated with low 5-HT levels, as indexed by 5-HT<sub>4</sub> receptor binding. *Social cognitive and affective neuroscience*. 2016 Apr; 11(4):548–55. <https://doi.org/10.1093/scan/nsv140> PMID: 26772668. Epub 2016/01/17. eng.
44. Knudsen GM, Jensen PS, Erritzoe D, Baare WF, Ettrup A, Fisher PM, et al. The Center for Integrated Molecular Brain Imaging (Cimbi) database. *Neuroimage*. 2016 Jan 1; 124(Pt B):1213–9. <https://doi.org/10.1016/j.neuroimage.2015.04.025> PMID: 25891375. Epub 2015/04/22. eng.
45. Madsen K, Haahr MT, Marner L, Svarer C, Hasselbalch SG, Knudsen GM, et al. Age and sex effects on 5-HT<sub>4</sub> receptors in the human brain: A 11 CSB207145 PET study. *Journal of Cerebral Blood Flow and Metabolism*. 2011; 31(6):1475–81. <https://doi.org/10.1038/jcbfm.2011.11> PMID: 21364600
46. Marner L, Gillings N, Madsen K, Erritzoe D, Baare WFC, Svarer C, et al. Brain imaging of serotonin 4 receptors in humans with [C-11]SB207145-PET. *Neuroimage*. 2010; 50(3):855–61. <https://doi.org/10.1016/j.neuroimage.2010.01.054> PMID: 20096787
47. Skovdahl HH, Mortensen EL, Schiøtz HK. NEO PI-R, manual—klinisk. 1. udgave, 5. oplag ed. Copenhagen: Hogrefe Psykologisk Forlag; 2011.
48. Olesen OV, Sibomana M, Keller SH, Andersen F, Jesnen JA, Holm S, et al., editors. Spatial resolution of the HRRT PET scanner using 4D-OSEM PSF reconstruction. *Nuclear Science Symposium Conference Record (NSS/MIC)*; 2009.
49. Svarer C, Madsen K, Hasselbalch SG, Pinborg LH, Haugbøl S, Frøkjær VG, et al. MR-based automatic delineation of volumes of interest in human brain PET images using probability maps. *Neuroimage*. 2005; 24(4):969–79. <https://doi.org/10.1016/j.neuroimage.2004.10.017> PMID: 15670674
50. Robert BI, Vincent JC, Jacques D, Masahiro F, Albert G, Roger NG, et al. Consensus nomenclature for in vivo imaging of reversibly binding radioligands. *Journal of Cerebral Blood Flow & Metabolism*. 2007; 27(9):1533.

51. Elliott R, Rubinsztein JS, Sahakian BJ, Dolan RJ. The neural basis of mood-congruent processing biases in depression. *Arch Gen Psychiatry*. 2002 Jul; 59(7):597–604. PMID: [12090812](#). Epub 2002/07/02. eng.
52. Holst KK, Budtz-Jørgensen E. Linear latent variable models: The lava-package. *Computational Statistics*. 2013; 28(4):1385–452.
53. Jones SE, Miller JD, Lynam DR. Personality, antisocial behavior, and aggression: A meta-analytic review. *Journal of Criminal Justice*. 2011 7//; 39(4):329–37.
54. Cloninger C, Svrakic DM, Przybeck TR. A psychobiological model of temperament and character. *Archives of General Psychiatry*. 1993; 50(12):975–90. PMID: [8250684](#)
55. Pinborg LH, Arfan H, Haugbol S, Kyvik KO, Hjelmborg JV, Svarer C, et al. The 5-HT2A receptor binding pattern in the human brain is strongly genetically determined. *Neuroimage*. 2008 Apr 15; 40(3):1175–80. <https://doi.org/10.1016/j.neuroimage.2007.09.019> PMID: [18291676](#). Epub 2008/02/23. eng.
56. Nielsen K, Brask D, Knudsen GM, Aznar S. Immunodetection of the serotonin transporter protein is a more valid marker for serotonergic fibers than serotonin. *Synapse (New York, NY)*. 2006 Apr; 59(5):270–6. <https://doi.org/10.1002/syn.20240> PMID: [16408260](#). Epub 2006/01/13. eng.
57. Gaspar P, Cases O, Maroteaux L. The developmental role of serotonin: news from mouse molecular genetics. *Nature reviews Neuroscience*. 2003 Dec; 4(12):1002–12. <https://doi.org/10.1038/nrn1256> PMID: [14618156](#). Epub 2003/11/18. eng.
58. Ansorge MS, Zhou M, Lira A, Hen R, Gingrich JA. Early-life blockade of the 5-HT transporter alters emotional behavior in adult mice. *Science*. 2004 Oct 29; 306(5697):879–81. <https://doi.org/10.1126/science.1101678> PMID: [15514160](#). Epub 2004/10/30. eng.
59. Domino G. *Psychological testing, an introduction*. 2. ed. ed. Cambridge New York Cambridge: Cambridge University Press; 2006.