Reduced serotonin receptors and transporters in normal aging adults: a meta-analysis of PET and SPECT imaging studies

Teresa M. Karrer [a, *], Casey L. McLaughlin [b], Carmela P. Guaglianone [c],

Gregory R. Samanez-Larkin [c, d, e]

[a] Department of Psychiatry, Psychotherapy and Psychosomatics, Faculty of Medicine, RWTH Aachen, Germany.

[b] Department of Biology, College of William and Mary, Williamsburg, VA, USA.

[c] Department of Psychology and Neuroscience, Duke University, Durham, NC, USA.

[d] Center for Cognitive Neuroscience, Duke University, Durham, NC, USA.

[e] Center for the Study of Aging and Human Development, Duke University, Durham, NC, USA.

* corresponding author: Teresa M. Karrer, M.Sc.

Email: tkarrer@ukaachen.de

Phone: +49 241 80-88734

Universitätsklinikum Aachen

Pauwelsstr. 30

52074 Aachen

GERMANY

Abstract:

Age-related alterations in serotonin (5-HT) function have been hypothesized to underlie a range of physiological, emotional, and cognitive changes in older age. Here, we conducted a quantitative synthesis and comparison of the effects of age on 5-HT receptors and transporters from cross-sectional PET and SPECT imaging studies. Random-effects meta-analyses of 31 studies including 1087 healthy adults yielded large negative age effects in 5-HT-2A receptors, moderate negative age effects in 5-HT transporters, and small negative age effects in 5-HT-1A receptors. Age differences were significantly larger in 5-HT-2A receptors compared to 5-HT-1A receptors. A meta-regression showed that 5-HT target, brain region, tracer kinetic measure, radionuclide, and publication year significantly moderated the age effect. The findings overall identify reduced serotonergic signal transmission in age. The evidence for a relative preservation of 5-HT-1A compared to 5-HT-2A receptors may partially explain differential psychological changes with age, such as why older people use more emotion-focused rather than problem-focused coping strategies.

Keywords: Meta-analysis; Serotonin; Healthy aging; Receptors; Transporters

1. Introduction

Serotonin (5-HT) is involved in a wide range of physiological, emotional, and cognitive functions (Lucki, 1998). The existence of at least 14 distinct receptor types that differ in their distribution across the brain might explain the functional diversity of the 5-HT system (Barnes and Sharp, 1999). Although the majority of studies of the functional consequences of alterations in the 5-HT system have focused on the role of 5-HT transporters in depression (Kambeitz and Howes, 2015; Ogilvie et al., 1996), 5-HT receptors have been specifically associated with several functions. For example, the inhibitory 5-HT-1A receptor has been linked to the sleep-wake cycle, the modulation of anxiety, and long-term memory, whereas the excitatory 5-HT-2A receptor has been associated with the modulation of hallucinogenic effects and both short and long-term memory (Gaspar et al., 2003; Hannon and Hoyer, 2008). Moreover, 5-HT dysfunctions in general have been implicated in several psychiatric and neurological diseases across the life span (Lucki, 1998) including age-related diseases like Alzheimer's disease (Blin et al 1993).

In contrast to candidate gene studies which make up the majority of individual difference studies of 5-HT (Caspi et al., 2010), positron emission tomography (PET) and single-photon emission computed tomography (SPECT) are brain imaging techniques that provide direct measures of 5-HT system function in-vivo. Over the last several decades, one of the most consistently observed individual difference in 5-HT using PET and SPECT imaging has been adult age-related decline. Studies of different receptor types, transporters, and synthesis capacity have revealed small to moderate negative age effects on 5-HT-1A receptors (Rabiner et al., 2002; Tauscher et al., 2001), moderate to extremely large age effects on 5-HT-2A receptors (Sheline et al., 2002; Uchida et al., 2011), and moderate to large effects of

age on 5-HT transporters (Fazio et al., 2016; Yamamoto et al., 2002) across adulthood. The only prior summary of the literature on 5-HT in aging, based primarily on post-mortem studies, concluded that 5-HT-1A and 5-HT-2A receptors decline with age (Meltzer et al., 1998). The evidence for age differences in 5-HT transporters was mixed, including findings of reduced, increased, and stable 5-HT transporter levels with age (Meltzer et al., 1998).

From neuroimaging studies, the previously reported sizes of adult age effects varied across 5-HT targets and cortical and subcortical brain regions. Some of the observed heterogeneities might also be due to sample variation in other variables of influence such as sex (Cidis Meltzer et al., 2001; Costes et al., 2005), body mass index (Hesse et al., 2014), hormone levels (Moses-Kolko et al., 2011), and genes (David et al., 2005). It is also possible that different 5-HT targets do not change uniformly across the life span but instead develop and age in a non-linear way. This might produce different estimates of age effects depending on the specific age ranges within each study. Furthermore, many PET and SPECT imaging studies lack appropriate sample sizes due to the high costs and safety concerns of the nuclear imaging technology. Thus, it is difficult to assess the reliability of a single effect from a single study.

Recent meta-analytic approaches have been used to aggregate and compare individual effects across studies. Although decades of research have documented age-related declines in neuromodulatory function in general, recent meta-analytic approaches have identified differential rates of decline across subcomponents of neuromodulatory systems. For example, a quantitative synthesis of age effects on the dopamine system in over 2000 participants revealed moderate to large age-related declines in D1- and D2-like receptors

and dopamine transporters (Karrer et al., 2017). Critically, the age effect in D1-like receptors was significantly larger than in D2-like receptors, whereas presynaptic synthesis capacity was preserved with age (Karrer et al., 2017). Although a qualitative summary of the existing neuroimaging literature suggests moderate to strong negative effects of age across the 5-HT system, human neuroimaging studies have not systematically and directly compared age effects across different 5-HT targets. Here, we used a meta-analytic approach to investigate variation in the effects of aging across the 5-HT system.

To quantitatively synthesize the existing literature on adult age differences in several targets of the 5-HT system, we conducted a comprehensive meta-analysis of cross-sectional PET and SPECT imaging studies. In light of previous individual study findings, we expected small to moderate negative age-related declines in 5-HT-1A receptors and moderate to large age-related declines in 5-HT-2A receptors and transporters. To investigate which factors might explain the heterogeneity of findings across previous studies, we examined the influence of potential moderating variables (e.g., age range, sex, imaging method, 5-HT target, brain region) in a meta-regression. In additional more exploratory analyses, we aggregated individual subject data across studies (where available) to examine the linearity of age effects across 5-HT targets. We did not make specific predictions for these exploratory analyses given the lack of prior reporting and low power of individual studies to examine these effects.

2. Methods

We conducted and reported this meta-analysis adhering to the guidelines of the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) statement (Liberarti

et al., 2009). Note that we did not pre-specify our approach in a review protocol. Instead, we closely followed the methods described in an earlier meta-analysis of our group on age differences in the dopamine system (Karrer et al., 2018). In addition, this facilitates the comparison of age effects across both neurochemical systems.

2.1 Literature search

Potentially relevant studies from the personal library of GRSL helped to define the keywords for the systematic literature search. We capitalized on the hierarchically organized vocabulary of Medical Subject Headings (MeSH) terms that enables a search for specific topics independent of the used words or spellings (Lipscomp, 2000). The MeSH terms “aging”, “emission computed tomography”, “humans”, “serotonin receptors”, “serotonin plasma membrane transport proteins”, “serotonin” and “synthesis” were combined to search the largest biomedical literature database PubMed (cf. Supplementary Materials for exact literature search terms). The last search was performed on 09/26/2018. To further complement the study corpus, we carefully checked the references of all relevant studies to search for additional includable studies.

We included all accessible PET and SPECT imaging studies of the 5-HT system written in English or German that (i) reported original results, (ii) contained a sample of healthy adults (> 18 years) with a minimum age range of 25 years to representatively measure the age effect, and (iii) reported or allowed for the computation of the Pearson correlation coefficient *r* between age and a 5-HT target. We included different tracer kinetic measures that quantify the functioning of 5-HT targets such as nondisplacable binding potential and binding potentials based on an arterial input function (Innis et al., 2007). We excluded

studies (i) if their sample primarily consisted of relatives of depressive patients because serotonergic alterations have been observed in this subgroup (Leboyer et al., 1999), (ii) if the reported age effects were adjusted for other variables such as sex (e.g., partial correlations and standardized slopes in multiple regression), and (iii) if their sample could be identified as subsample of another included study. To avoid duplicates in the included subjects, we thoroughly inspected sample descriptions and compared experimental characteristics across studies.

2.2 Data extraction

Given that many studies reported the Pearson correlation coefficient *r* between age and individual targets of the 5-HT system (receptors, transporters, synthesis capacity), we used this standardized statistic in all meta-analytic analyses. The included studies either directly reported or enabled the computation of the Pearson correlation coefficient *r* from (i) a table, (ii) a figure (using PlotDigitizer version 2.6.8), or (iii) the group effect of age in older versus younger adults. We summarized the age effects for each of the following brain regions: brainstem and diencephalon (midbrain, pons, and medulla, hypothalamus and thalamus), medial temporal lobe (amygdala, hippocampus, parahippocampal gyrus, entorhinal cortex, and perirhinal cortex), cingulate, basal ganglia (putamen, caudate, ventral striatum, and pallidum), insula, occipital, parietal, temporal, frontal and global cortex. If a study reported the age effect separately for males and females, right and left hemisphere, or several sub-regions of the brain, we averaged the correlation coefficients using Fisher's *z* transformation. Particularly in small samples, this conversion to an approximately normally distributed measure introduces less bias in the computation of the mean (Silver and Dunlap, 1987). If a study computed the age effects using several nuclear imaging analysis methods, we chose

the method which the authors referred to as gold-standard or which was most commonly used in the literature.

In addition to the effect sizes of primary interest (*r* between 5-HT target and age), we extracted the following data: sample size, age range, female percent, imaging method, scanner model, 5-HT target, brain region, radiotracer, tracer kinetic measure, reference region, and partial volume correction. To examine the nature of the age effects in more detail, we extracted age and 5-HT target data of individual subjects from tables or figures reported in a majority of the included studies. All extracted data are available at osf.io/3897j.

2.3 Data analysis

First, we examined the possibility that studies reporting stronger effects were more likely to get published and thus, included in the meta-analyses. To qualitatively assess publication bias, we used contour-enhanced funnel plots. In contrast to the traditional version, this visual tool indicates areas of statistical significance. This approach distinguishes publication bias from other types of biases such as study quality (Peters et al., 2008). We additionally applied the rank correlation test (Begg and Mazumdar, 1994) to quantitatively test for publication bias.

The primary goal was to meta-analytically summarize the correlations between age and different 5-HT targets across studies. We estimated one meta-analysis for each 5-HT target split into several target regions. Because the studies were based on different populations and varied in many aspects, we chose random-effects models to account for the

heterogeneity between studies (Borenstein et al., 2010). In case the meta-analyses relied on too few studies to robustly estimate the heterogeneity between studies, we additionally compared the results to fixed-effect models, which only assume variance within the studies (Borenstein et al., 2010). If a study provided age correlations for more than one region, we used a multi-variate meta-analysis model to account for the statistical dependence of the effects sizes (Jackson et al., 2011). To stabilize the variances of the effect sizes, Pearson's correlation coefficients *r* were converted to Fisher's *z* before analysis (e.g., Viechtbauer, 2010).

In the next step, we examined how much of the overall study heterogeneity could be attributed to differences between studies rather than errors within studies using Q and I^2 statistics (Higgins et al., 2003). To investigate which variables might explain the observed heterogeneity between the studies, we used a mixed-effects meta-regression. We included the following potential moderator variables in the meta-regression: age range and female percent of the sample, publication year, imaging method, radionuclide, tracer kinetic measure, reference region, partial volume correction, 5-HT target, and brain region (Table S1). We centered continuous variables first to facilitate interpretation. Since some studies contributed several effect sizes to the meta-regression, we again used a multivariate model that accounts for the statistical dependence of the effect sizes.

Because the study-level meta-analyses of linear age effects were limited in providing insight into potential nonlinear associations between age and 5-HT targets, we additionally analyzed individual subject data. We estimated the type of relationship between age and different 5-HT targets using raw data extracted from a subset of the studies. If a study reported the

tracer kinetic measure values for several brain regions instead of a global aggregation, we averaged the tracer kinetic measure values in each subject first. Then, we z-scored the individual tracer kinetic measure values in each study to allow for a comparison across different tracer kinetic measures. Finally, we fit linear, quadratic, and logarithmic functions to the individual data points to investigate which model best described the relationship between age and 5-HT targets. In addition, the extracted raw data enabled us to estimate the percent change per decade for different 5-HT targets. For this purpose, we divided the slope of the linear function by the range of the tracer kinetic measure values and multiplied the proportion by 10.

Finally, the meta-analytically-derived summary effect sizes were used to conduct statistical power analyses. Based on a two-sided significance level of *alpha* = 0.05, we determined the percentage of included studies with at least 80% statistical power. In addition, we computed the necessary sample sizes to detect age effects in different 5-HT targets, which could be useful for future studies.

Analyses were conducted in R (version 3.4.0) primarily using the metafor package (Viechtbauer, 2010). All data analysis scripts are publicly available at osf.io/ctn83 for transparency and reuse.

3. Results

3.1 Literature search

The literature search yielded 298 potentially relevant studies. The study selection process is detailed in a PRISMA flowchart (Figure S1). Studies reporting age effects in 5-HT-1B

receptors (Matuskey et al., 2012; Nord et al., 2014), 5-HT-4 receptors (Madsen et al., 2011; Marner et al., 2010), and 5-HT synthesis capacity (Rosa-Neto et al., 2007) were excluded due to an insufficient number to conduct a meta-analysis. A total of 31 studies reporting age effects in 5-HT-1A receptors (n=8), 5-HT-2A receptors (n=18), and 5-HT transporters (n=6) were included in the meta-analyses (cf. Supplementary Materials for the reference list; one study examined age effects in two 5-HT targets (Moses-Kolko et al., 2011)). The study corpus included 83.9% PET studies, 35.1% females, and a mean age range of 49 years in 1087 healthy adults (Table S2).

3.2 Publication bias and meta-analysis

The funnel plots contained few non-significant studies in 5-HT-2A receptors and 5-HT transporters, which could indicate publication bias (Figure S2). However, the applied rank correlation tests did not detect any significant relationship between effect sizes and sampling variance in 5-HT-2A receptors (*Kendall's* τ = -0.21*;* p = 0.078) and in 5-HT transporters (*Kendall's* τ = *-0.07;* p = 1.0). In 5-HT-1A receptors, however, the rank correlation test yielded a significant funnel plot asymmetry (*Kendall's* τ = -0.38*;* p = 0.004). The random-effects meta-analyses estimated the summary correlation coefficients and their 95% confidence intervals. The average age effect was r = –0.25 [–0.4; –0.08] in 5-HT-1A receptors (Figure 1), r = –0.71 [–0.77; –0.63] in 5-HT-2A receptors (Figure 2), and r = –0.47 [–0.63; –0.27] in 5-HT transporters (Figure 3). Different variants of univariate and fixed-effect meta-analytic models yielded comparable results (Table S3). In addition, we computed the summary effect sizes separately for different brain regions (Table 1). The comparison of the 95% confidence intervals revealed a significantly larger age-related decline in 5-HT-2A receptors compared to 5-HT-1A receptors (Figure 4).

3.3 Heterogeneity and meta-regression

Significant *Q*-statistics in 5-HT-1A receptors (Q = 46.8%; p = 0.045), 5-HT-2A receptors (Q = 68.7%; p = 0.001), and 5-HT transporters (Q = 15.3%; p = 0.009) indicated that the included studies did not share a common effect size. Similarly, the I^2-statistics revealed moderate amounts of heterogeneity in 5-HT-1A receptors (I^2 = 70.0%), 5-HT-2A receptors (I^2 = 57.4%), and 5-HT transporters (I^2 = 68.6%). The mixed-effects meta-regression showed that the included variables explained a significant amount of the observed heterogeneity in the age effect ($p < 0.001$). Publication year ($p < 0.001$), tracer kinetic measure (p = 0.04), and radionuclide (p = 0.01) emerged as significant moderators of the age effect (Table 2). Specifically, smaller age effects were reported in more recent publications, studies reporting a binding potential based on arterial input functions instead of a nondisplacable binding potential, and studies using the radiotracer ^{11}C instead of ^{18}F. Post-hoc tests, which evaluated the influence of a variable across several factor levels, additionally showed significant moderating effects of 5-HT target (p = 0.013) and brain region (p = 0.025). Age range, female percent, imaging method, reference region, and partial volume correction did not significantly moderate the age effect. A test for residual heterogeneity indicated that other variables not considered in the meta-regression did not significantly influence the age effect (p = 0.217).

3.4 Linearity/non-linearity of age effects and percent change per decade

Individual subject data for at least a subset of brain regions were available for nearly all of the studies (n=29 out of 31). This allowed us to test linear, quadratic, and logarithmic effects of age across 5-HT targets (Figure 5). Linear age effects explained 2% of the variance in 5-HT-

1A receptors ($p < 0.05$), 41.9% of variance in 5-HT-2A receptors ($p < 0.001$) and 11.7% of variance in 5-HT transporters ($p < 0.001$). The fits of the quadratic and logarithmic functions were comparable to those of the linear function while adjusting for model complexity (Table S4). The quadratic term, however, did not add a significant amount of explained variance in any of the three 5-HT targets ($p_{5\text{-}HT\text{-}1A} = 0.976$; $p_{5\text{-}HT\text{-}2A} = 0.726$; $p_{5\text{-}HT} = 0.934$).

Using linear models, the observed percent declines per decade were 1.5% in 5-HT-1A receptors, 7.3% in 5-HT-2A receptors, and 3.0% in 5-HT transporters. The rank order of these effects is consistent with the average age correlations yielded from the meta-analyses. These measures are provided as a more concrete quantification of age-related differences relative to 5-HT functioning in younger adulthood.

3.5 Statistical power analyses

The average sample sizes of the included studies were about 35 subjects (*median* = 22), ranging from 7 to 132 subjects. Assuming that the meta-analytic effect sizes closely resembled the true age effect, we found an average power of 71.4% percent across studies. Half of the studies reached a statistical power of at least 80%, which is often recommended. The power analyses further revealed the minimum sample sizes required for a minimum of 80% of statistical power in detecting linear age effects at 123 subjects for 5-HT-1A receptors, 13 subjects for 5-HT-2A receptors, and 33 subjects for 5-HT transporters.

4. Discussion

The results of the present meta-analysis of in-vivo PET and SPECT imaging studies in healthy adults revealed several negative effects of age on serotonergic functioning. Consistent with

our hypotheses, we found large, negative age effects in 5-HT-2A receptors, moderate, negative age effects in 5-HT transporters, and small, negative age effects in 5-HT-1A receptors. Overall, the age-related declines across 5-HT targets ranged between 1.5% and 7.3% per decade. In addition to variation in age effects across 5-HT targets and brain regions, we identify further variables that moderated age effects. Overall, the study provides the first quantitative synthesis of adult age differences in the 5-HT system.

The meta-analytic evidence for fewer postsynaptic receptors implies a restricted capacity to transmit 5-HT mediated signals in older age. This alteration in signal transduction might be compensated by two mechanisms whereby 5-HT could be active in the synaptic cleft for a longer time interval. On the one hand, the activity of synaptic 5-HT could be prolonged due to the lower number of 5-HT transporters in age. The presynaptic transporter proteins may limit clearance of 5-HT from the synaptic cleft via re-uptake into the presynaptic neuron. On the other hand, the activity period of synaptic 5-HT could be lengthier because of a lower number of presynaptic 5-HT-1A auto-receptors in age. Hence, this negative feedback process might be restricted in the inhibition of 5-HT release into the synaptic cleft. To corroborate and complement these preliminary insights into serotonergic functioning in the elderly, future studies could investigate how age affects other receptor types and presynaptic mechanisms such as synthesis capacity. Unfortunately there were not enough studies of age differences in synthesis capacity to meta-analyze. One of the few studies on 5-HT synthesis capacity reported stable levels of this presynaptic 5-HT target across the adult life span (Rosa-Neto et al., 2007).

5-HT-1A and 5-HT-2A receptors differ in their distribution across the brain and signaling cascades (Hannon and Hoyer, 2008). For instance, electrophysiological experiments have shown that 5-HT-1A receptor activation induces a hyperpolarization (Nicoll et al., 1990), whereas 5-HT-2A receptor activation primarily leads to a depolarization of the postsynaptic neuron (Aghajanian, 1995). Hence, both receptor systems likely mediate distinct functions in the brain (Barnes and Sharp, 1999). A recent theory suggests that 5-HT-1A and 5-HT-2A receptors enable humans to adapt flexibly to adverse events in the environment (Carhart-Harris and Nutt, 2017). Concretely, the mostly inhibitory 5-HT-1A receptor was assumed to mediate passive coping responses such as internal reappraisal of a stressful situation (Carhart-Harris and Nutt, 2017). The mainly excitatory 5-HT-2A receptor however, was suggested to mediate active coping strategies such as engaging in an interpersonal confrontation (Carhart-Harris and Nutt, 2017). In light of this hypothesis, the finding of stronger age-related declines in 5-HT-2A receptors compared to 5-HT-1A receptors might be viewed as consistent with the evidence that older adults use more passive, emotion-focused than active, problem-focused coping strategies (Folkman et al., 1987). The potential mediation of these behavioral age differences by serotonergic function has not been directly tested. Surprisingly, few studies (n=6) examined associations between 5HT receptors and transporters and physiological, emotional, and cognitive variables, primarily focusing on personality traits (Frokjaer et al., 2009; Parsey et al., 2002; Rosell et al., 2010) and hormone levels (Frokjaer et al., 2009; Moses-Kolko et al., 2011). Thus, additional research is needed to examine the functional consequences of these neurobiological changes with age.

Besides variation across 5-HT targets and brain regions, we observed that the age effects were moderated by publication year, tracer kinetic measure, and radionuclide. The evidence

that smaller age effects were reported in more recently published studies might be explained by higher effective camera resolutions due to improved technologies and more advanced quantification methods. These newer cameras may better assess individual subregions of the brain and minimize signal mixing with nearby white matter or cerebrospinal fluid (see below for additional discussion of partial volume effects). Unfortunately, we were not able to further investigate this hypothesis since effective scanner resolution is only rarely reported in PET and SPECT imaging studies. Secondly, different age effects in studies using nondisplacable binding potentials versus tracer kinetic measures based on arterial input functions could arise from the difficulty to measure certain radioligands in plasma because of their fast clearance rate from blood (Gunn et al., 1998). Thirdly, the moderating effect of radionuclide might be accounted for by the more reliable and accurate signal of ^{18}F because of its longer half-life and shorter positron range compared to ^{11}C (Morris et al., 2013). An important caveat here is not to over-interpret the results of the meta-regression. The moderating effects could also be explained by shared confounds between the studies. For instance, we were not able to consider previously reported moderators of the age effect such as body mass index (Hesse et al., 2014), sex hormones (Moses-Kolko et al., 2011), and genes (David et al., 2005). Furthermore, we did not find any evidence for a moderating effect of sex as some studies have reported previously (e.g., Baeken et al., 1998; Cidis Meltzer et al., 2001). Yet, this finding is in line with studies based on larger samples that did also not find an age by sex interaction (e.g., Frokjaer et al., 2009; van Dyck et al., 2000).

A further limitation of the meta-analysis is the potential overestimation of the age effects. One reason might be publication bias as quantitatively indicated in 5-HT-1A receptors and

informally observed during the literature search. A few studies reporting a non-significant age effect did not state the exact size of the age effect and could hence not be included in the meta-analyses. This is a major limitation of the study. Partial volume effects could have additionally contributed to an overestimation of the age effects in the 5-HT system. There is considerable evidence for grey matter density reductions in age (Sowell et al., 2003). PET and SPECT signals in atrophy-affected, smaller brain regions are more susceptible to blurring by surrounding tissue (Hoffman et al., 1979; Hoffman et al., 1982). Such partial volume effects can lead to an underestimation of the 5-HT signal in age, as has been reported in the dopamine system (Morris et al., 1999; Smith et al., 2017). Several established techniques are available to account for partial volume effects (e.g., Meltzer et al., 1990; Muller-Gartner et al., 1992; Rousset et al., 1998), but only 7 out of 31 included studies applied such corrections. The potential underestimation of the 5-HT signal in a majority of the included studies might have led to an overestimation of the age effect in the meta-analyses. Yet, we did not find any evidence for a systematic influence of partial volume corrections on the reported age effects. To differentiate age-related receptor loss from age-related neuronal atrophy, it will be important for future studies to directly compare age effects in uncorrected data and after correcting for partial volume effects.

The present study has several additional limitations, both at the level of the meta-analyses and the level of the included studies. First, the literature search was only based on one database as opposed to the recommendations of the PRISMA statement. Yet, we inspected a large number of potentially relevant studies and conducted a thorough reference search. Second, the individual studies seemed to involve on average a lower number of older adults than younger adults as indicated by the scatter plots. This imbalance might explain why we

did not find clear evidence for nonlinear changes of the 5-HT system across adulthood. Third, the individual studies used radionuclides of varying quality. We attempted to include only studies using radionuclides of sufficient affinity and selectivity for 5-HT (Paterson et al., 2013). Finally, we only included cross-sectional studies whereby the reported age effects could potentially be biased by cohort effects. The only longitudinal study so far showed stable 5-HT-2A receptor levels after two years but suffered from a small sample size and a limited age range (Marner et al., 2009). Hence, longitudinal studies with sufficient power and a broad age range will be necessary to disentangle age and cohort effects.

Here we conducted the first meta-analysis that precisely quantifies adult age differences in several 5-HT targets. The finding of an age-related reduction in 5-HT receptors reveals consistent evidence for limitations in serotonergic signal transmission in older age. Age-related losses in the 5HT system might be compensated by fewer presynaptic 5-HT auto-receptors and 5-HT transporters, which might lead to a prolonged activity of 5-HT in the synaptic cleft. The evidence for a relative preservation of 5-HT-1A compared to 5-HT-2A receptors connects a recent theory on 5-HT functioning to behavioral evidence for age differences in the use of coping strategies. Finally, to complement the suggestions for future research, the meta-analytic effects were used to estimate required minimum sample sizes in future studies of age differences in 5HT function.

Conflict of interest

The authors declare no competing financial interests.

Acknowledgements

TMK was supported by the International Research Training Group (IRTG 2150) of the German Research Foundation and by a full doctoral scholarship of the German National Academic foundation. GRSL was supported by US National Institute on Aging Pathway to Independence Award R00-AG042596.

References

Aghajanian, G.K., 1995. Electrophysiology of serotonin receptor subtypes and signal transduction pathways., in: Bloom, F.R., Kupfer, D.J. (Eds.), Psychopharmacology: The Fourth Generation of Progress. Raven, New York, pp. 1451-1459.

Baeken, C., D'Haenen, H., Flamen, P., Mertens, J., Terriere, D., Chavatte, K., Boumon, R., Bossuyt, A., 1998. 123I-5-I-R91150, a new single-photon emission tomography ligand for 5-HT2A receptors: influence of age and gender in healthy subjects. Eur. J. Nucl. Med. 25(12), 1617-1622.

Barnes, N.M., Sharp, T., 1999. A review of central 5-HT receptors and their function. Neuropharmacology 38(8), 1083-1152.

Begg, C.B., Mazumdar, M., 1994. Operating characteristics of a rank correlation test for publication bias. Biometrics 50(4), 1088-1101.

Borenstein, M., Hedges, L.V., Higgins, J.P., Rothstein, H.R., 2010. A basic introduction to fixed-effect and random-effects models for meta-analysis. Research synthesis methods 1(2), 97-111.

Carhart-Harris, R.L., Nutt, D.J., 2017. Serotonin and brain function: a tale of two receptors. Journal of psychopharmacology (Oxford, England) 31(9), 1091-1120.

Caspi, A., Hariri, A.R., Holmes, A., Uher, R., Moffitt, T.E., 2010. Genetic sensitivity to the environment: the case of the serotonin transporter gene and its implications for studying complex diseases and traits. Am. J. Psychiatry 167(5), 509-527.

Cidis Meltzer, C., Drevets, W.C., Price, J.C., Mathis, C.A., Lopresti, B., Greer, P.J., Villemagne, V.L., Holt, D., Mason, N.S., Houck, P.R., Reynolds, C.F., 3rd, DeKosky, S.T., 2001. Gender-specific aging effects on the serotonin 1A receptor. Brain Res. 895(1-2), 9-17.

Costes, N., Merlet, I., Ostrowsky, K., Faillenot, I., Lavenne, F., Zimmer, L., Ryvlin, P., Le Bars, D., 2005. A 18F-MPPF PET normative database of 5-HT1A receptor binding in men and women over aging. J. Nucl. Med. 46(12), 1980-1989.

Cumming, G., 2009. Inference by eye: reading the overlap of independent confidence intervals. Stat. Med. 28, 205-220.

David, S.P., Murthy, N.V., Rabiner, E.A., Munafo, M.R., Johnstone, E.C., Jacob, R., Walton, R.T., Grasby, P.M., 2005. A functional genetic variation of the serotonin (5-HT) transporter affects 5-HT1A receptor binding in humans. J. Neurosci. 25(10), 2586-2590.

Fazio, P., Schain, M., Varnas, K., Halldin, C., Farde, L., Varrone, A., 2016. Mapping the distribution of serotonin transporter in the human brainstem with high-resolution PET: Validation using postmortem autoradiography data. Neuroimage 133, 313-320.

Folkman, S., Lazarus, R.S., Pimley, S., Novacek, J., 1987. Age differences in stress and coping processes. Psychol. Aging 2(2), 171-184.

Frokjaer, V.G., Erritzoe, D., Madsen, J., Paulson, O.B., Knudsen, G.M., 2009. Gender and the use of hormonal contraception in women are not associated with cerebral cortical 5-HT 2A receptor binding. Neuroscience 163(2), 640-645.

Gaspar, P., Cases, O., Maroteaux, L., 2003. The developmental role of serotonin: news from mouse molecular genetics. Nat. Rev. Neurosci. 4(12), 1002-1012.

Gunn, R.N., Sargent, P.A., Bench, C.J., Rabiner, E.A., Osman, S., Pike, V.W., Hume, S.P., Grasby, P.M., Lammertsma, A.A., 1998. Tracer kinetic modeling of the 5-HT1A receptor ligand [carbonyl-11C]WAY-100635 for PET. Neuroimage 8(4), 426-440.

Hannon, J., Hoyer, D., 2008. Molecular biology of 5-HT receptors. Behav. Brain Res. 195(1), 198-213.

Hesse, S., van de Giessen, E., Zientek, F., Petroff, D., Winter, K., Dickson, J.C., Tossici-Bolt, L., Sera, T., Asenbaum, S., Darcourt, J., Akdemir, U.O., Knudsen, G.M., Nobili, F., Pagani, M., Vander Borght, T., Van Laere, K., Varrone, A., Tatsch, K., Sabri, O., Booij, J., 2014. Association of central serotonin transporter availability and body mass index in healthy Europeans. Eur. Neuropsychopharmacol. 24(8), 1240-1247.

Higgins, J.P., Thompson, S.G., Deeks, J.J., Altman, D.G., 2003. Measuring inconsistency in meta-analyses. BMJ 327(7414), 557-560.

Hoffman, E.J., Huang, S.C., Phelps, M.E., 1979. Quantitation in positron emission computed tomography: 1. Effect of object size. J. Comput. Assist. Tomogr. 3(3), 299-308.

Hoffman, E.J., Huang, S.C., Plummer, D., Phelps, M.E., 1982. Quantitation in positron emission computed tomography: 6. effect of nonuniform resolution. J. Comput. Assist. Tomogr. 6(5), 987-999.

Innis, R.B., Cunningham, V.J., Delforge, J., Fujita, M., Gjedde, A., Gunn, R.N., Holden, J., Houle, S., Huang, S.-C., Ichise, M., Iida, H., Ito, H., Kimura, Y., Koeppe, R.A., Knudsen, G.M., Knuuti, J., Lammertsma, A.A., Laruelle, M., Logan, J., Maguire, R.P., Mintun, M.A., Morris, E.D.W., Dean F, Parsey, R., Price, J.C., Slifstein, M., Sossi, V., Suhara, T., Votaw, J.R., Wong, D.F., Carson, R.E., 2007. Consensus nomenclature for in vivo imaging of reversibly binding radioligands. J. Cereb. Blood Flow Metab. 27, 1533-1539.

Jackson, D., Riley, R., White, I.R., 2011. Multivariate meta-analysis: potential and promise. Stat. Med. 30(20), 2481-2498.

Kambeitz, J.P., Howes, O.D., 2015. The serotonin transporter in depression: Meta-analysis of in vivo and post mortem findings and implications for understanding and treating depression. J. Affect. Disord. 186, 358-366.

Karrer, T.M., Josef, A.K., Mata, R., Morris, E.D., Samanez-Larkin, G.R., 2017. Reduced dopamine receptors and transporters but not synthesis capacity in normal aging adults: a meta-analysis. Neurobiol. Aging 57, 36-46.

Leboyer, M., Quintin, P., Manivet, P., Varoquaux, O., Allilaire, J.-F., Launay, J.-M., 1999. Decreased serotonin transporter binding in unaffected relatives of manic depressive patients. Biol. Psychiatry 46(12), 1703-1706.

Liberarti, A., Altman, D.G., Tetzlaff, J., Mulrow, C., Gotzsche, P.C., Ioannidis, J.P.A., Clarke, M., Devereaux, P.J., Kleinjen, J., Moher, D., 2009. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate healthcare interventions: explanation and elaboration. The BMJ 339, 1-27.

Lipscomp, C.E., 2000. Medical Subject Headings (MeSH). Bull. Med. Libr. Assoc. 88, 265-266.

Lucki, I., 1998. The spectrum of behaviors influenced by serotonin. Biol. Psychiatry 44(3), 151-162.

Madsen, K., Haahr, M.T., Marner, L., Keller, S.H., Baare, W.F., Svarer, C., Hasselbalch, S.G., Knudsen, G.M., 2011. Age and sex effects on 5-HT(4) receptors in the human brain: a [(11)C]SB207145 PET study. J. Cereb. Blood Flow Metab. 31(6), 1475-1481.

Marner, L., Gillings, N., Madsen, K., Erritzoe, D., Baare, W.F., Svarer, C., Hasselbalch, S.G., Knudsen, G.M., 2010. Brain imaging of serotonin 4 receptors in humans with [11C]SB207145-PET. Neuroimage 50(3), 855-861.

Marner, L., Knudsen, G.M., Haugbol, S., Holm, S., Baare, W., Hasselbalch, S.G., 2009. Longitudinal assessment of cerebral 5-HT2A receptors in healthy elderly volunteers: an [18F]-altanserin PET study. Eur. J. Nucl. Med. Mol. Imaging 36(2), 287-293.

Matuskey, D., Pittman, B., Planeta-Wilson, B., Walderhaug, E., Henry, S., Gallezot, J.D., Nabulsi, N., Ding, Y.S., Bhagwagar, Z., Malison, R., Carson, R.E., Neumeister, A., 2012. Age effects on serotonin receptor 1B as assessed by PET. J. Nucl. Med. 53(9), 1411-1414.

Meltzer, C.C., Leal, J.P., Mayberg, H.S., Wagner, H.N., Jr., Frost, J.J., 1990. Correction of PET data for partial volume effects in human cerebral cortex by MR imaging. J. Comput. Assist. Tomogr. 14(4), 561-570.

Meltzer, C.C., Smith, G., DeKosky, S.T., Pollock, B.G., Mathis, C.A., Moore, R.Y., Kupfer, D.J., Reynolds, C.F., 3rd, 1998. Serotonin in aging, late-life depression, and Alzheimer's disease: the emerging role of functional imaging. Neuropsychopharmacology 18(6), 407-430.

Morris, E.D., Chefer, S.I., Lane, M.A., Muzic, R.F., Jr., Wong, D.F., Dannals, R.F., Matochik, J.A., Bonab, A.A., Villemagne, V.L., Grant, S.J., Ingram, D.K., Roth, G.S., London, E.D., 1999. Loss of D2 receptor binding with age in rhesus monkeys: importance of correction for differences in striatal size. J. Cereb. Blood Flow Metab. 19(2), 218-229.

Morris, E.D., Lucas, M.V., Cosgrove, K.P., 2013. How to study smoking and drinking with PET, Positron Emission Tomography - Recent Developments in Instrumentation, Research and Clinical Oncological Practice al. InTech, pp. 103-149.

Moses-Kolko, E.L., Price, J.C., Shah, N., Berga, S., Sereika, S.M., Fisher, P.M., Coleman, R., Becker, C., Mason, N.S., Loucks, T., Meltzer, C.C., 2011. Age, sex, and reproductive hormone effects on brain serotonin-1A and serotonin-2A receptor binding in a healthy population. Neuropsychopharmacology 36(13), 2729-2740.

Muller-Gartner, H.W., Links, J.M., Prince, J.L., Bryan, R.N., McVeigh, E., Leal, J.P., Davatzikos, C., Frost, J.J., 1992. Measurement of radiotracer concentration in brain gray matter using positron emission tomography: MRI-based correction for partial volume effects. J. Cereb. Blood Flow Metab. 12(4), 571-583.

Nicoll, R.A., Malenka, R.C., Kauer, J.A., 1990. Functional comparison of neurotransmitter receptor subtypes in mammalian central nervous system. Physiol. Rev. 70(2), 513-565.

Nord, M., Cselenyi, Z., Forsberg, A., Rosenqvist, G., Tiger, M., Lundberg, J., Varrone, A., Farde, L., 2014. Distinct regional age effects on [11C]AZ10419369 binding to 5-HT1B receptors in the human brain. Neuroimage 103, 303-308.

Ogilvie, A.D., Battersby, S., Bubb, V.J., Fink, G., Harmar, A.J., Goodwim, G.M., Smith, C.A., 1996. Polymorphism in serotonin transporter gene associated with susceptibility to major depression. Lancet 347(9003), 731-733.

Parsey, R.V., Oquendo, M.A., Simpson, N.R., Ogden, R.T., Van Heertum, R., Arango, V., Mann, J.J., 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(2), 173-182.

Paterson, L.M., Kornum, B.R., Nutt, D.J., Pike, V.W., Knudsen, G.M., 2013. 5-HT radioligands for human brain imaging with PET and SPECT. Med. Res. Rev. 33(1), 54-111.

Peters, J.L., Sutton, A.J., Jones, D.R., Abrams, K.R., Rushton, L., 2008. Contour-enhanced meta-analysis funnel plots help distinguish publication bias from other causes of asymmetry. J. Clin. Epidemiol. 61(10), 991-996.

Rabiner, E.A., Messa, C., Sargent, P.A., Husted-Kjaer, K., Montgomery, A., Lawrence, A.D., Bench, C.J., Gunn, R.N., Cowen, P., Grasby, P.M., 2002. 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 15(3), 620-632.

Rosa-Neto, P., Benkelfat, C., Sakai, Y., Leyton, M., Morais, J.A., Diksic, M., 2007. Brain regional alpha-[11C]methyl-L-tryptophan trapping, used as an index of 5-HT synthesis, in healthy adults: absence of an age effect. Eur. J. Nucl. Med. Mol. Imaging 34(8), 1254-1264.

Rosell, D.R., Thompson, J.L., Slifstein, M., Xu, X., Frankle, W.G., New, A.S., Goodman, M., Weinstein, S.R., Laruelle, M., Abi-Dargham, A., Siever, L.J., 2010. Increased serotonin 2A receptor availability in the orbitofrontal cortex of physically aggressive personality disordered patients. Biol. Psychiatry 67(12), 1154-1162.

Rousset, O.G., Ma, Y., Evans, A.C., 1998. Correction for partial volume effects in PET: principle and validation. Journal of Nuclear Medicine Med 39, 904-911.

Sheline, Y.I., Mintun, M.A., Moerlein, S.M., Snyder, A.Z., 2002. Greater loss of 5-HT(2A) receptors in midlife than in late life. Am. J. Psychiatry 159(3), 430-435.

Silver, N.C., Dunlap, W.P., 1987. Averaging correlation coefficients: should Fisher's z transformation be used? J. Appl. Psychol. 72, 146-148.

Smith, C.T., Crawford, J.L., Dang, L.C., Seaman, K.L., San Juan, M.D., Vijay, A., Katz, D.T., Matuskey, D., Cowan, R.L., Morris, E.D., Zald, D.H., Samanez-Larkin, G.R., 2017. Partial-volume correction increases estimated dopamine D2-like receptor binding potential and reduces adult age differences. J. Cereb. Blood Flow Metab., 271678x17737693.

Sowell, E.R., Peterson, B.S., Thompson, P.M., Welcome, S.E., Henkenius, A.L., Toga, A.W., 2003. Mapping cortical change across the human life span. Nat. Neurosci. 6(3), 309-315.

Tauscher, J., Verhoeff, N.P., Christensen, B.K., Hussey, D., Meyer, J.H., Kecojevic, A., Javanmard, M., Kasper, S., Kapur, S., 2001. Serotonin 5-HT1A receptor binding potential

declines with age as measured by [11C]WAY-100635 and PET. Neuropsychopharmacology 24(5), 522-530.

Uchida, H., Chow, T.W., Mamo, D.C., Kapur, S., Mulsant, B.H., Houle, S., Pollock, B.G., Graff-Guerrero, A., 2011. Effects of aging on 5-HT(2A) R binding: a HRRT PET study with and without partial volume corrections. Int. J. Geriatr. Psychiatry 26(12), 1300-1308.

van Dyck, C.H., Malison, R.T., Seibyl, J.P., Laruelle, M., Klumpp, H., Zoghbi, S.S., Baldwin, R.M., Innis, R.B., 2000. Age-related decline in central serotonin transporter availability with [(123)I]beta-CIT SPECT. Neurobiol. Aging 21(4), 497-501.

Viechtbauer, W., 2010. Conducting meta-analyses in R with the metafor package. Journal of Statistical Software 36, 1-48.

Yamamoto, M., Suhara, T., Okubo, Y., Ichimiya, T., Sudo, Y., Inoue, M., Takano, A., Yasuno, F., Yoshikawa, K., Tanada, S., 2002. Age-related decline of serotonin transporters in living human brain of healthy males. Life Sci. 71(7), 751-757.

Figures

Figure 1

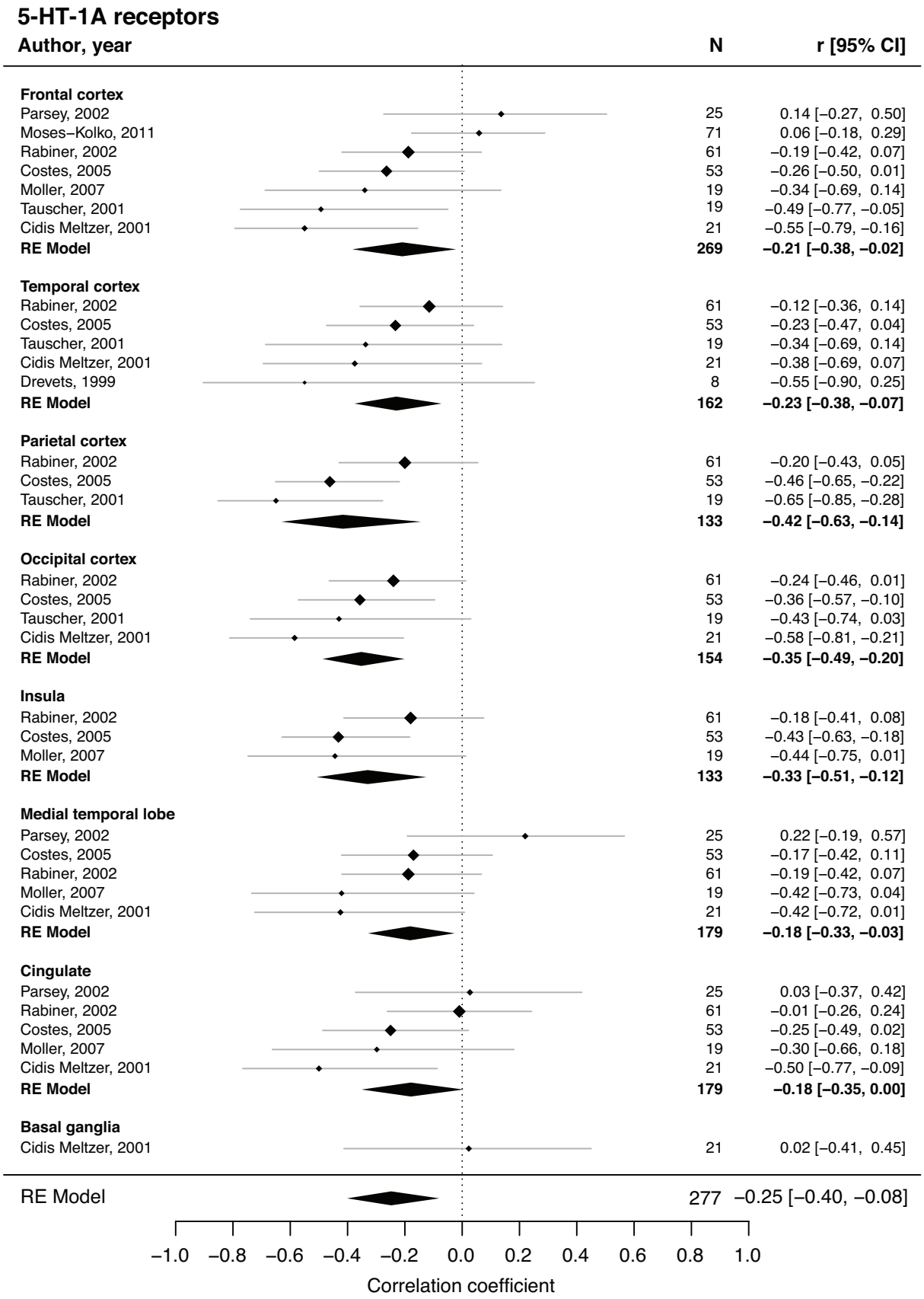


Fig. 1. Forest plot of age effects in 5-HT-1A receptors. Depicts reported effect size (diamond) and 95% confidence interval (line) for each included study. Weight of each study (size of diamond) depends on sample size and between-study variance. Summary effects sizes (polygon) and 95% confidence interval (width of polygon) for separate brain regions and in total. N represents the number of individual subjects included in each study and in the computation of the summary effect sizes.

Figure 2

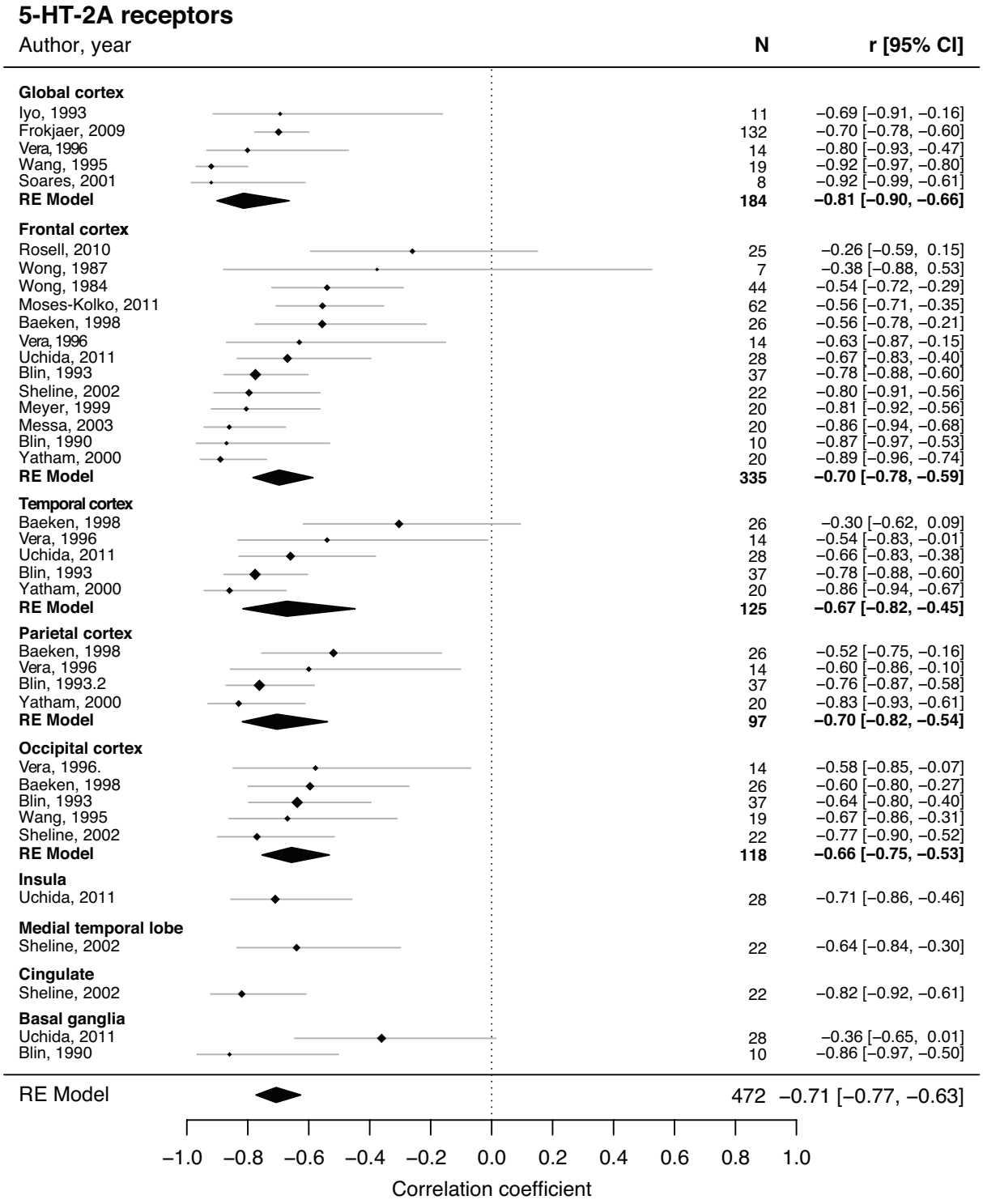


Fig. 2. Forest plot of age effects in 5-HT-2A receptors. Depicts reported effect size (diamond) and 95% confidence interval (line) for each included study. Weight of each study (size of diamond) depends on sample size and between-study variance. Summary effects sizes

(polygon) and 95% confidence interval (width of polygon) for separate brain regions and in total. N represents the number of individual subjects included in each study and in the computation of the summary effect sizes.

Figure 3

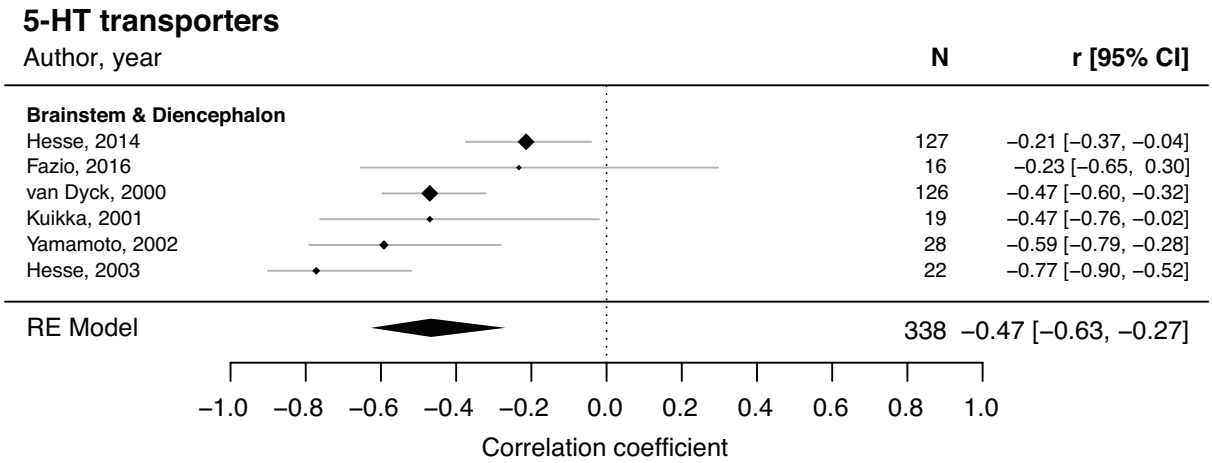


Fig. 3. Forest plot of age effects in 5-HT transporters. Depicts reported effect size (diamond) and 95% confidence interval (line) for each included study. Weight of each study (size of diamond) depends on sample size and between-study variance. Summary effects sizes (polygon) and 95% confidence interval (width of polygon) for separate brain regions and in total. N represents the number of individual subjects included in each study and in the computation of the summary effect sizes.

Figure 4

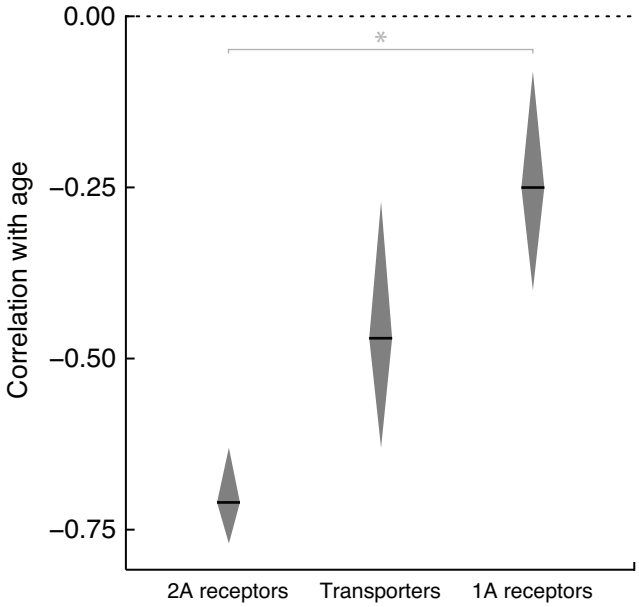


Fig. 4. Comparison of age effects in 5-HT-1A receptors, 5-HT-2A receptors, and 5-HT transporters. Black line indicates summary effect size and height of polygon represents 95% confidence interval. Dotted line marks an age effect of zero. Difference between age-related declines in 5-HT-1A receptors and 5-HT-2A receptor was statistically significant at $p < 0.01$ (Cumming, 2009).

Figure 5

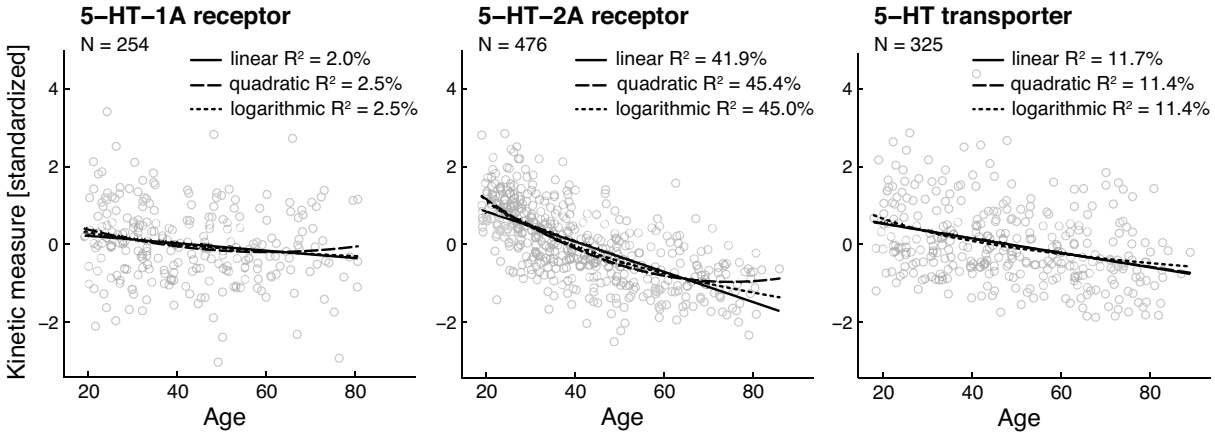


Fig. 5. Scatter plots depicting the relationship between age and 5-HT targets. Linear, quadratic, and logarithmic model were fit to individual age and standardized tracer kinetic measure data in 5-HT-1A receptors, 5-HT-2A receptors, and 5-HT transporters. N indicates the number of individual subjects included in the analysis. R^2 values are adjusted R^2.

Tables

Table 1

Results of meta-analyses of adult age differences in 5-HT-1A receptors, 5-HT-2A receptors, and 5-HT transporters.

Target	r	95% CI		# of studies	# of subjects
5-HT-1A receptor	-0.25	-0.4	-0.08	8	277
Frontal cortex	-0.21	-0.38	-0.02	7	269
Temporal cortex	-0.23	-0.38	-0.07	5	162
Parietal cortex	-0.42	-0.63	-0.14	3	133
Occipital cortex	-0.35	-0.49	-0.2	4	154
Insula	-0.33	-0.51	-0.12	3	133
Medial temporal lobe	-0.18	-0.33	-0.03	5	179
Cingulate	-0.18	-0.35	0	5	179
5-HT-2A receptor	-0.71	-0.77	-0.63	17	472
Global cortex	-0.81	-0.9	-0.66	5	184
Frontal cortex	-0.7	-0.78	-0.59	13	335
Temporal cortex	-0.67	-0.82	-0.45	5	125
Parietal cortex	-0.7	-0.82	-0.54	4	97
Occipital cortex	-0.66	-0.75	-0.53	5	118
5-HT transporter	-0.47	-0.63	-0.27	6	338

Table 2

Influence of potential moderators on the correlation between age and 5-HT targets in a multi-variate mixed-effects meta-regression.

Moderator variable (factor level)	**r**	**SE**	**95% CI**	
Intercept	-0,25	0,13	-0,46	-0,01
Age range	0	0,01	-0,01	0,01
Female percent	-0,17	0,31	-0,65	0,41
Publication year	0,02	0,01	0,01	0,04
Imaging method (SPECT)	0,19	0,25	-0,29	0,59
Tracer kinetic measure (BP_{ai})	0,28	0,13	0,02	0,5
Radionuclide (^{18}F)	-0,41	0,16	-0,64	-0,12
Reference region (occipital)	0,23	0,27	-0,29	0,64
Partial volume correction (Applied)	0,02	0,15	-0,26	0,3
Target (5-HT transporter)	-0,57	0,23	-0,8	-0,18
Target (5-HT-2A receptor)	-0,29	0,16	-0,55	0,02
Region (Basal ganglia)	0,33	0,17	0,01	0,59
Region (Cingulate)	-0,03	0,11	-0,24	0,18
Region (Frontal cortex)	-0,09	0,09	-0,27	0,08
Region (Global cortex)	-0,47	0,21	-0,73	-0,09
Region (Insula)	-0,15	0,11	-0,35	0,06
Region (Medial temporal lobe)	0	0,11	-0,21	0,2
Region (Occipital cortex)	-0,14	0,1	-0,33	0,06
Region (Parietal cortex)	-0,17	0,11	-0,36	0,03

Note: Three studies missing information were automatically dropped from the meta-regression. The factor level "^{123}I" of the radionuclide variable was automatically dropped from the model due to a complete redundancy with the factor level "SPECT" of the imaging method variable.

Supplementary Materials
for
Reduced serotonin receptors and transporters in normal aging adults: a meta-analysis of PET and SPECT imaging studies

Teresa M. Karrer [a, *], Casey L. McLaughlin [b], Carmela P. Guaglianone [c], Gregory R. Samanez-Larkin [c, d, e]

[a] Department of Psychiatry, Psychotherapy and Psychosomatics, Faculty of Medicine, RWTH Aachen, Germany.
[b] Department of Biology, College of William and Mary, Williamsburg, VA, USA.
[c] Department of Psychology and Neuroscience, Duke University, Durham, NC, USA.
[d] Center for Cognitive Neuroscience, Duke University, Durham, NC, USA.
[e] Center for the Study of Aging and Human Development, Duke University, Durham, NC, USA.

* corresponding author. Email: tkarrer@ukaachen.

Table of Content

Literature search terms 2
Supplementary Table 1 3
Supplementary Figure 1 4
References of included studies 5
Supplementary Table 2 8
Supplementary Figure 2 9
Supplementary Table 3 10
Supplementary Table 4 11

Literature search terms

a) 5-HT receptors (n=103)

```
(„Aging"[Mesh] OR "Aged"[Mesh]) AND ("tomography, emission-computed" [Mesh]) AND "receptors, Serotonin"[Mesh] AND "humans" [Mesh]
```

b) 5-HT transporters (n=66)

```
(„Aging"[Mesh] OR "Aged"[Mesh]) AND ("tomography, emission-computed" [Mesh]) AND "serotonin plasma membrane transport proteins"[Mesh] AND "humans" [Mesh]
```

c) 5-HT synthesis capacity (n=60)

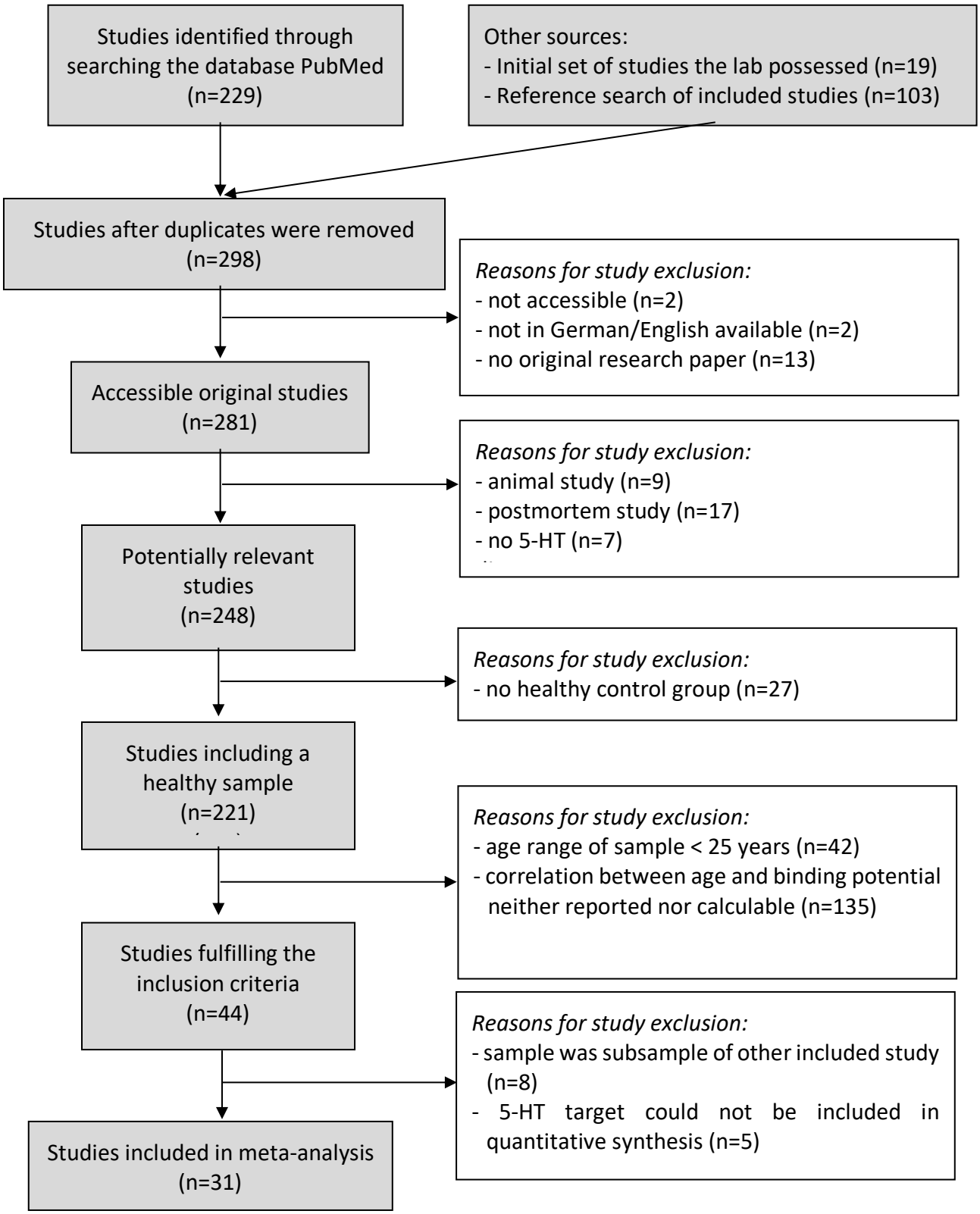
```
(„Aging"[Mesh] OR "Aged"[Mesh]) AND ("tomography, emission-computed" [Mesh]) AND serotonin[Mesh] AND synthesis AND "humans" [Mesh]
```

Supplementary Table 1
Variables tested as potential moderators in a mixed-effects model.

Continuous variables	
Age range (centered)	
Female percent (centered)	
Publication year (centered)	
Discrete variables	**Factor levels**
Imaging method	PET
	SPECT
Tracer kinetic measure	Binding potential with arterial input function (BP_{ai})
	Nondisplacable binding potential (BP_{nd})
Radionuclide	^{11}C
	^{18}F
	^{123}I
Reference region	Cerebellum
	Occipital cortex
Partial volume correction	Applied
	Not applied
Target	5-HT-1A receptor
	5-HT-2A receptor
	5-HT transporter
Region	Brainstem & Diencephalon
	Basal ganglia
	Cingulate
	Medial temporal lobe
	Insula
	Occipital cortex
	Parietal cortex
	Temporal cortex
	Frontal cortex
	Global cortex

Note: In discrete variables, the factor level listed first represents the reference level used in the moderator analysis.

Supplementary Figure 1



SFig. 1. Flow diagram of study selection for the quantitative meta-analyses.

References of included studies

Baeken, C., D'Haenen, H., Flamen, P., Mertens, J., Terriere, D., Chavatte, K., … Bossuyt, A. (1998). 123I-5-I-R91150, a new single-photon emission tomography ligand for 5-HT2A receptors: influence of age and gender in healthy subjects. *European Journal of Nuclear Medicine*, *25*(12), 1617–1622.

Blin, J., Baron, J. C., Dubois, B., Crouzel, C., Fiorelli, M., Attar-Levy, D., … Agid, Y. (1993). Loss of brain 5-HT2 receptors in Alzheimer's disease. In vivo assessment with positron emission tomography and [18F]setoperone. *Brain*, *116 (Pt 3)*, 497–510.

Blin, J., Sette, G., Fiorelli, M., Bletry, O., Elghozi, J. L., Crouzel, C., & Baron, J. C. (1990). A method for the in vivo investigation of the serotonergic 5-HT2 receptors in the human cerebral cortex using positron emission tomography and 18F-labeled setoperone. *Journal of Neurochemistry*, *54*(5), 1744–1754.

Cidis Meltzer, C., Drevets, W. C., Price, J. C., Mathis, C. A., Lopresti, B., Greer, P. J., … DeKosky, S. T. (2001). Gender-specific aging effects on the serotonin 1A receptor. *Brain Research*, *895*(1–2), 9–17.

Costes, N., Merlet, I., Ostrowsky, K., Faillenot, I., Lavenne, F., Zimmer, L., … Le Bars, D. (2005). A 18F-MPPF PET normative database of 5-HT1A receptor binding in men and women over aging. *Journal of Nuclear Medicine*, *46*(12), 1980–1989.

Drevets, W. C., Frank, E., Price, J. C., Kupfer, D. J., Holt, D., Greer, P. J., … Mathis, C. (1999). PET imaging of serotonin 1A receptor binding in depression. *Biological Psychiatry*, *46*(10), 1375–1387.

Fazio, P., Schain, M., Varnas, K., Halldin, C., Farde, L., & Varrone, A. (2016). Mapping the distribution of serotonin transporter in the human brainstem with high-resolution PET: Validation using postmortem autoradiography data. *Neuroimage*, *133*, 313–320.

Frokjaer, V. G., Erritzoe, D., Madsen, J., Paulson, O. B., & Knudsen, G. M. (2009). Gender and the use of hormonal contraception in women are not associated with cerebral cortical 5-HT 2A receptor binding. *Neuroscience*, *163*(2), 640–645.

Hesse, S., Barthel, H., Murai, T., Muller, U., Muller, D., Seese, A., … Sabri, O. (2003). Is correction for age necessary in neuroimaging studies of the central serotonin transporter? *European Journal of Nuclear Medicine and Molecular Imaging*, *30*(3), 427–430.

Hesse, S., van de Giessen, E., Zientek, F., Petroff, D., Winter, K., Dickson, J. C., … Booij, J. (2014). Association of central serotonin transporter availability and body mass index in healthy Europeans. *European Neuropsychopharmacology*, *24*(8), 1240–1247.

Iyo, M., & Yamasaki, T. (1993). The detection of age-related decrease of dopamine D1, D2 and serotonin 5-HT2 receptors in living human brain. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *17*(3), 415–421.

Kuikka, J. T., Tammela, L., Bergstrom, K. A., Karhunen, L., Uusitupa, M., & Tiihonen, J. (2001). Effects of ageing on serotonin transporters in healthy females. *European Journal of Nuclear Medicine*, *28*(7), 911–913.

Messa, C., Colombo, C., Moresco, R. M., Gobbo, C., Galli, L., Lucignani, G., … Fazio, F. (2003). 5-HT(2A) receptor binding is reduced in drug-naive and unchanged in SSRI-responder depressed patients compared to healthy controls: a PET study. *Psychopharmacology*, *167*(1), 72–78.

Meyer, J. H., Kapur, S., Houle, S., DaSilva, J., Owczarek, B., Brown, G. M., … Kennedy, S. H. (1999). Prefrontal cortex 5-HT2 receptors in depression: an [18F]setoperone PET imaging study. *American Journal of Psychiatry*, *156*(7), 1029–1034.

Mintun, M. A., Sheline, Y. I., Moerlein, S. M., Vlassenko, A. G., Huang, Y., & Snyder, A. Z. (2004). Decreased hippocampal 5-HT2A receptor binding in major depressive disorder: in vivo measurement with [18F]altanserin positron emission tomography. *Biological Psychiatry*, *55*(3), 217–224.

Moller, M., Jakobsen, S., & Gjedde, A. (2007). Parametric and regional maps of free serotonin 5HT1A receptor sites in human brain as function of age in healthy humans. *Neuropsychopharmacology*, *32*(8), 1707–1714.

Moses-Kolko, E. L., Price, J. C., Shah, N., Berga, S., Sereika, S. M., Fisher, P. M., … Meltzer, C. C. (2011). Age, sex, and reproductive hormone effects on brain serotonin-1A and serotonin-2A receptor binding in a healthy population. *Neuropsychopharmacology*, *36*(13), 2729–2740.

Parsey, R. V, Oquendo, M. A., Simpson, N. R., Ogden, R. T., Van Heertum, R., Arango, V., & Mann, J. J. (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 Research*, *954*(2), 173–182.

Rabiner, E. A., Messa, C., Sargent, P. A., Husted-Kjaer, K., Montgomery, A., Lawrence, A. D., … Grasby, P. M. (2002). 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*, *15*(3), 620–632.

Rosell, D. R., Thompson, J. L., Slifstein, M., Xu, X., Frankle, W. G., New, A. S., … Siever, L. J. (2010). Increased serotonin 2A receptor availability in the orbitofrontal cortex of physically aggressive personality disordered patients. *Biological Psychiatry*, *67*(12), 1154–1162.

Sheline, Y. I., Mintun, M. A., Moerlein, S. M., & Snyder, A. Z. (2002). Greater loss of 5-HT(2A) receptors in midlife than in late life. *American Journal of Psychiatry*, *159*(3), 430–435.

Soares, J. C., van Dyck, C. H., Tan, P., Zoghbi, S. S., Garg, P., Soufer, R., … Innis, R. B. (2001). Reproducibility of in vivo brain measures of 5-HT2A receptors with PET and. *Psychiatry Research*, *106*(2), 81–93.

Tauscher, J., Verhoeff, N. P., Christensen, B. K., Hussey, D., Meyer, J. H., Kecojevic, A., … Kapur, S. (2001). Serotonin 5-HT1A receptor binding potential declines with age as measured by [11C]WAY-100635 and PET. *Neuropsychopharmacology*, *24*(5), 522–530.

Uchida, H., Chow, T. W., Mamo, D. C., Kapur, S., Mulsant, B. H., Houle, S., … Graff-Guerrero, A. (2011). Effects of aging on 5-HT(2A) R binding: a HRRT PET study with and without partial volume corrections. *International Journal of Geriatric Psychiatry*, *26*(12), 1300–1308.

van Dyck, C. H., Malison, R. T., Seibyl, J. P., Laruelle, M., Klumpp, H., Zoghbi, S. S., … Innis, R. B. (2000). Age-related decline in central serotonin transporter availability with [(123)I]beta-CIT SPECT. *Neurobiology of Aging*, *21*(4), 497–501.

Vera, P., Zilbovicius, M., Chabriat, H., Amarenco, P., Kerdraon, J., Menard, J. F., … Samson, Y. (1996). Post-stroke changes in cortical 5-HT2 serotonergic receptors. *Journal of Nuclear Medicine*, *37*(12), 1976–1981.

Wang, G. J., Volkow, N. D., Logan, J., Fowler, J. S., Schlyer, D., MacGregor, R. R., … Wolf, A. P. (1995). Evaluation of age-related changes in serotonin 5-HT2 and dopamine D2 receptor availability in healthy human subjects. *Life Sciences*, *56*(14), Pl249-53.

Wong, D. F., Lever, J. R., Hartig, P. R., Dannals, R. F., Villemagne, V., Hoffman, B. J., … et al. (1987). Localization of serotonin 5-HT2 receptors in living human brain by positron emission tomography using N1-([11C]-methyl)-2-Br-LSD. *Synapse*, *1*(5), 393–398.

Wong, D. F., Wagner Jr., H. N., Dannals, R. F., Links, J. M., Frost, J. J., Ravert, H. T., … et al. (1984). Effects of age on dopamine and serotonin receptors measured by positron tomography in the living human brain. *Science*, *226*(4681), 1393–1396.

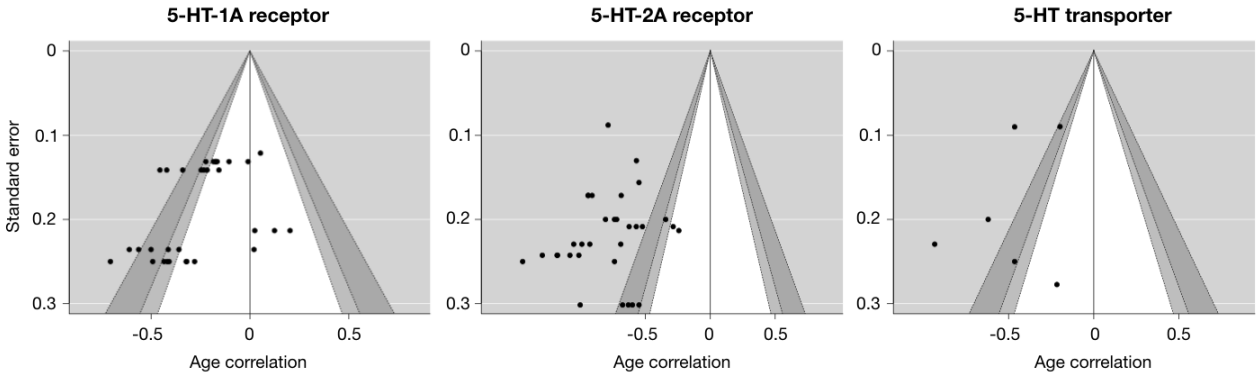
Yamamoto, M., Suhara, T., Okubo, Y., Ichimiya, T., Sudo, Y., Inoue, M., … Tanada, S. (2002). Age-related decline of serotonin transporters in living human brain of healthy males. *Life Sciences*, *71*(7), 751–757.

Yatham, L. N., Liddle, P. F., Shiah, I. S., Scarrow, G., Lam, R. W., Adam, M. J., … Ruth, T. J. (2000). Brain serotonin2 receptors in major depression: a positron emission tomography study. *Archives of General Psychiatry*, *57*(9), 850–858.

Supplementary Table 2
Characteristics of the studies included in the meta-analyses.

First author	Publication Year	N	Age minimum	Age maximum	Female in %	Imaging method	Target
Baeken	1998	26	23	60	50	SPECT	5HT-2A receptor
Blin	1990	10	20	74	0	PET	5HT-2A receptor
Blin	1993	37	20	86	-	PET	5HT-2A receptor
Cidis Meltzer	2001	21	21	80	52.4	PET	5HT-1A receptor
Costes	2005	53	20	70	50.9	PET	5HT-1A receptor
Drevets	1999	8	-	-	50	PET	5HT-1A receptor
Fazio	2016	16	21	67	14.3	PET	5HT transporter
Frokjaer	2009	132	18	79	34.1	PET	5HT-2A receptor
Hesse	2003	22	18	83	63.6	SPECT	5HT transporter
Hesse	2014	127	20	83	45.7	SPECT	5HT transporter
Iyo	1993	11	22	72	9.1	PET	5HT-2A receptor
Kuikka	2001	19	22	74	100	SPECT	5HT transporter
Messa	2003	20	22	59	45	PET	5HT-2A receptor
Meyer	1999	20	18	45	57.9	PET	5HT-2A receptor
Mintun	2004	29	22	79	69	PET	5HT-2A receptor
Moller	2007	19	23	73	42.1	PET	5HT-1A receptor
Moses-Kolko	2011	71	20	80	48.6	PET	5HT-1A receptor & 5HT-2A receptor
Parsey	2002	25	20	70	48	PET	5HT-1A receptor
Rabiner	2002	61	24	53	0	PET	5HT-1A receptor
Rosell	2010	25	19	55	40	PET	5HT-2A receptor
Sheline	2002	22	21	69	72.7	PET	5HT-2A receptor
Soares	2001	8	19	69	25	PET	5HT-2A receptor
Tauscher	2001	19	22	53	42.1	PET	5HT-1A receptor
Uchida	2011	28	19	78	46.4	PET	5HT-2A receptor
van Dyck	2000	126	18	88	44.4	SPECT	5HT transporter
Vera	1996	14	30	72	-	PET	5HT-2A receptor
Wang	1995	19	21	49	0	PET	5HT-2A receptor
Wong	1984	44	19	73	50	PET	5HT-2A receptor
Wong	1987	7	28	68	0	PET	5HT-2A receptor
Yamamoto	2002	28	20	79	0	PET	5HT transporter
Yatham	2000	20	19	59	60	PET	5HT-2A receptor
31 studies	2001.3	35.1	21	70	40	26 PET studies	1087 individuals

Supplementary Figure 2



SFig. 2. Funnel plots illustrating potential publication bias. Depict distribution of included studies in relation to the size of the reported effect (x-axis) and sample size (y-axis) in 5-HT-1A receptors, 5-HT-2A receptors, and 5-HT transporters. The shaded regions correspond to different levels of statistical significance: $p > 0.1$ (white region); $0.1 > p > 0.4$ (grey region); $p < 0.01$ (dark-grey region). Centered at zero (no age effect).

Supplementary Table 3
Comparison of different meta-analytic models examining the age effect on 5-HT targets.

Target	Model	r	95% CI	
5-HT-1A receptor	univariate fixed-effect model	–0.24	–0.29	–0.18
	multivariate fixed-effect model	–0.24	–0.29	–0.18
	univariate random-effects model	–0.25	–0.32	–0.18
	multivariate random-effects model	–0.25	–0.4	–0.08
5-HT-2A receptor	univariate fixed-effect model	–0.69	–0.72	–0.66
	multivariate fixed-effect model	–0.69	–0.72	–0.66
	univariate random-effects model	–0.7	–0.75	–0.65
	multivariate random-effects model	–0.71	–0.77	–0.63
5-HT transporter	univariate fixed-effect model	–0.4	–0.49	–0.31
	multivariate fixed-effect model	–0.4	–0.49	–0.31
	univariate random-effects model	–0.47	–0.63	–0.27
	multivariate random-effects model	–0.47	–0.63	–0.27

Supplementary Table 4
Linear, quadratic, and logarithmic models fit to individual standardized tracer kinetic measure and age data.

Target	Model	slope	p	R^2	p	Adj. R^2	# of subjects
5-HT-1A receptor	Linear model	–0,01	0,013	0,024	0,013	0,02	254
	Quadratic model	1,447	0,976	0,033	0,016	0,025	254
	Logarithmic model	–0,435	0,007	0,028	0,007	0,025	254
5-HT-2A receptor	Linear model	–0,039	< 0.001	0,42	< 0.001	0,419	476
	Quadratic model	4,11	0,726	0,457	< 0.001	0,454	476
	Logarithmic model	–1,703	< 0.001	0,451	< 0.001	0,45	476
5-HT transporter	Linear model	–0,019	< 0.001	0,12	< 0.001	0,117	325
	Quadratic model	0,14	0,934	0,12	< 0.001	0,114	325
	Logarithmic model	–0,821	< 0.001	0,117	< 0.001	0,114	325