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Structural and functional cerebral changes in patients with schizophrenia and genetic risk-allele carriers

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Summary

Schizophrenia is one of the most frequent psychiatric disorders and is associated with a substantial part of worldwide disease burdon[1]. The clinical symptoms of patients with schizophrenia can be separated into positive symptoms such as halluciations and delusions as well as negative symptoms such as cognitive impairments, apathy, blunted affect and social withdrawal[2]. It has been suggested that understanding the underlying pathophysiological processes that give rise to these symptoms is a crucial step for the development of efficient treatment for schizophrenia[3]. In the presented work two aspects of the clinical symptomatology of schizophrenia are analyzed with respect to their potential neurobiological correlate.

Following the dopamine-hypothesis, patients with schizophrenia exhibit an increase in dopaminergic neurotransmission in the striatum which might be related to the experience of positive symptoms[4,5]. In the first publication evidence for this dopamine-hypothesis from in-vivo neuroimaging studies was investigated in a comprehensive meta-analysis. Results are in the line with the dopamine-hypothesis and point to an increase of striatal presynaptic dopamine synthesis in schizophrenia:

- ***Howes OD*, Kambeitz J*, Kim E, Stahl D, Slifstein M, Abi-Dargham A*, Kapur S* (2012): The nature of dopamine dysfunction in schizophrenia and what this means for treatment. Arch Gen Psychiatry 69: 776–786. * these authors contributed equally***

  ISI Web of Knowledge: Archives of General Psychiatry (now: JAMA Psychiatry)
  impact factor 2012: 13.77
  5-year impact factor 2012: 14.47
  Ranked 3rd of all psychiatry journals

The negative symptoms of schizophrenia such as cognitive impairments have frequently been associated with changes of cerebral gray matter in numerous brain regions including the hippocampus[6–9]. In the second publication, effects of a potential risk-gene on the hippocampus are analyzed. Results indicate reduced hippocampal structure and function in carriers of the met-allele of the BDNF polymorphism val(66)met:

- ***Kambeitz JP*, Bhattacharyya S*, Kambeitz-Ilankovic LM, Valli I, Collier DA, McGuire P (2012): Effect of BDNF val(66)met polymorphism on declarative memory and its***

neural substrate: a meta-analysis. Neurosci Biobehav Rev 36: 2165–2177. * these authors contributed equally

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Zusammenfassung

Schizophrenie ist einer der häufigsten psychiatrischen Erkrankungen und verantwortlich für einen substanziellen Anteil der weltweiten Gesundheitsbelastung[1]. Die klinischen Symptome bei Patienten mit Schizophrenie werden eingeteilt in Positivsymptomatik wie Halluzinationen und Wahnvorstellungen sowie Negativsymptomatik wie kognitive Beeinträchtigungen, Apathie, verflachter Affekt und sozialer Rückzug[2]. Die Aufklärung der pathophysiologischen Prozesse welche der Entstehung von Positiv- und Negativsymptomatik zu Grunde liegen, ist ein entscheidender Schritt um effiziente pharmakologisch Behandlung für Patienten bieten zu können[3]. In der vorgestellten Arbeit sollen zwei Aspekte der klinischen Symptomatik schizophrener Patienten im Bezug auf deren neurobiologischen Korrelate analysiert werden.

Nach der Dopamin-Hypothese zeigen Patienten mit paranoider Schizophrenie eine gesteigerte dopaminerge Neurotransmission im Striatum[4,5]. Dieser hyperdopaminerge Zustand ist steht möglicherweise mit Positivsymptomatik in Verbindung[4]. In der ersten Publikation wurde die Evidenz für die Dopamin-Hypothese aus in-vivo Neuroimagingstudien im Rahmen einer Meta-Analyse überprüft werden. Die Ergebnisse unterstützen die Dopamin-Hypothese und sprechen für eine gesteigerte präsynaptische Dopamin-Synthese:

- ***Howes OD*, Kambeitz J*, Kim E, Stahl D, Slifstein M, Abi-Dargham A*, Kapur S* (2012): The nature of dopamine dysfunction in schizophrenia and what this means for treatment. Arch Gen Psychiatry 69: 776–786. * these authors contributed equally***

  ISI Web of Knowledge: Archives of General Psychiatry (now: JAMA Psychiatry)
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Die Negativsymptomatik wie zum Beispiel kognitive Beeinträchtigungen wurden mit strukturellen cerebralen Veränderungen schizophrener Patienten in Verbindung gebracht - insbesondere des Hippocampus[6–9]. Im Rahmen der zweiten Publikation wurde der Effekt eines Risikogens auf den Hippocampus analysiert. Die Ergebnisse zeigen eine reduzierte hippocampale Struktur und Funktion sowie reduzierte Gedächtnisleistung bei Trägern des Risiko-Allels:

- ***Kambeitz JP*, Bhattacharyya S*, Kambeitz-Ilankovic LM, Valli I, Collier DA, McGuire P (2012): Effect of BDNF val(66)met polymorphism on declarative memory and its neural substrate: a meta-analysis. Neurosci Biobehav Rev 36: 2165–2177. * these authors contributed equally***

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List of abbreviations

BDNF	brain-derived neurotrophic factor
DAT	dopamine transporter
LTP	long-term potentiation
LTD	long-term depression
met	methionine
MRI	magnetic resonance imaging
fMRI	functional magnetic resonance imaging
SNP	single-nucleotide polymorphism
val	valine

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Introduction

Schizophrenia: epidemiology, clinical symptoms and pathophysiology

With a prevalence of 1 % in the general population schizophrenia is one of the main factors of global disease burdon[1]. The disorder is associated with severe consequences for the individual patients, their relatives as well as society. Schizophrenia usually onsets in early adulthood and on average affects women as frequently as men.

The clinical picture of schizophrenia is variable and there is no clear core symptomatology that is present in all cases and distinguishes schizophrenia from other mental disorders.

Typically symptoms of schizophrenia are classified into positive and negative symptoms[2]. Positive symptoms include hallucinations, delusions, thought disorders as well as disorganized behaviour. Negative symptoms include cognitive impairments, apathy, blunted affect, social withdrawal and self neglect.

Despite substantial research effort to disentangle the pathophysiology of schizophrenia, the specific causes remain unknown. Until today no single pathphysiological account exists that could explain all the findings in a conclusive manner. However some aspects of the rich symptomatology have been related to biological changes found in patients.

In the present work two important concepts of the pathophysiology of schizophrenia are investigated. The first concept suggests a relationsship between positive symptoms and abnormal dopaminergic neurotransmission in the striatum of patients with schizophrenia. This theory is refered to as the dopamine-hypothesis of schizophrenia and is supported by substantial evidence from multiple lines of research[4]. Three different aspects of striatal dopaminergic neurotransmission are summarized in a separate meta-analysis. Implications for our understanding of the pathophysiology of schizophrenia as well as for treatment of affected patients are discussed.

The second concept focusses on decreased memory as an example of cognitive impairment in patients with schizophrenia. It is suggested that carriers of a specific risk-allele might exhibit altered hippocampal structure and function which might in turn result in impaired memory performance. In three separate meta-analyses the effect of a genetic polymorphism in the gene of the brain-derived neurotrophic factor (BDNF) on hippocampal structure and function is investigated.

The dopamine hypothesis

The dopamine-hypothesis was originally based on indirect evidence such as the psychotogenic effect of dopaminergic substances. For instance amphetamines, which increase the extracellular concentration of dopamine, have been shown to induce psychotic symptoms in patients with schizophrenia[10]. More direct evidence for the involvement of dopamine in the pathogenesis of schizophrenia was provided by the early investigation of dopamine antagonists as antipsychotic medication[11,12]. It was demonstrated that these drugs bind to the post-synaptic dopamine receptor to block dopaminergic neurotransmission which results in a reduction of symptoms in schizophrenic patients. Moreover, a relationship between the receptor affinity of antipsychotic drugs and their antipsychotic potency has been reported[13].

These findings lead to the formulation of the dopamine hypothesis as a „receptor hypothesis“[14]. It was stated that an increase of postsynaptic dopamine receptors might be the cause of schizophrenia and that it could be treated with postsynaptic receptor blockade.

In a central article by Davis et al.[5] the dopamine hypothesis was reformulated to include the current evidence at that time. Studies of cerebrospinal fluid as well as post-mortem brain tissue samples did not support the notion of an overall increase of dopaminergic neurotransmission in patients. In addition early neuroimaging studies in humans pointed to a hypometabolism in cortical areas of schizophrenic patients which was not well explained by a general excess of dopamine. Instead of a general hyperdopaminergic state, Davis et al. claimed an imbalance in schizophrenia with subcortical *hyper*dopaminergia and cortical *hypo*dopaminergia. It was suggested that cortical decrease of dopaminergic neurotransmission could be associated with negative symptoms while subcortical and in particular striatal increase of dopaminergic neurotransmission might be associated with positive symptoms.

In a third and most recent formulation of the dopamine hypothesis, Howes & Kapur[4] further specified the location of striatal hyperdopaminergia and suggested a link to genetic factors, environmental factors as well as clinical symptoms. The in-vivo investigation of changes dopaminergic neurotransmission in schizophrenia has become feasible with the introduction of new radioligands that specifically bind to different molecular structures. Current results show an increase of presynaptic dopamine synthesis[15–19]. Most interestingly this increase is already present in patients in the prodromal stage of psychosis[20,21] or in first-degree relatives of schizophrenic patients[22].

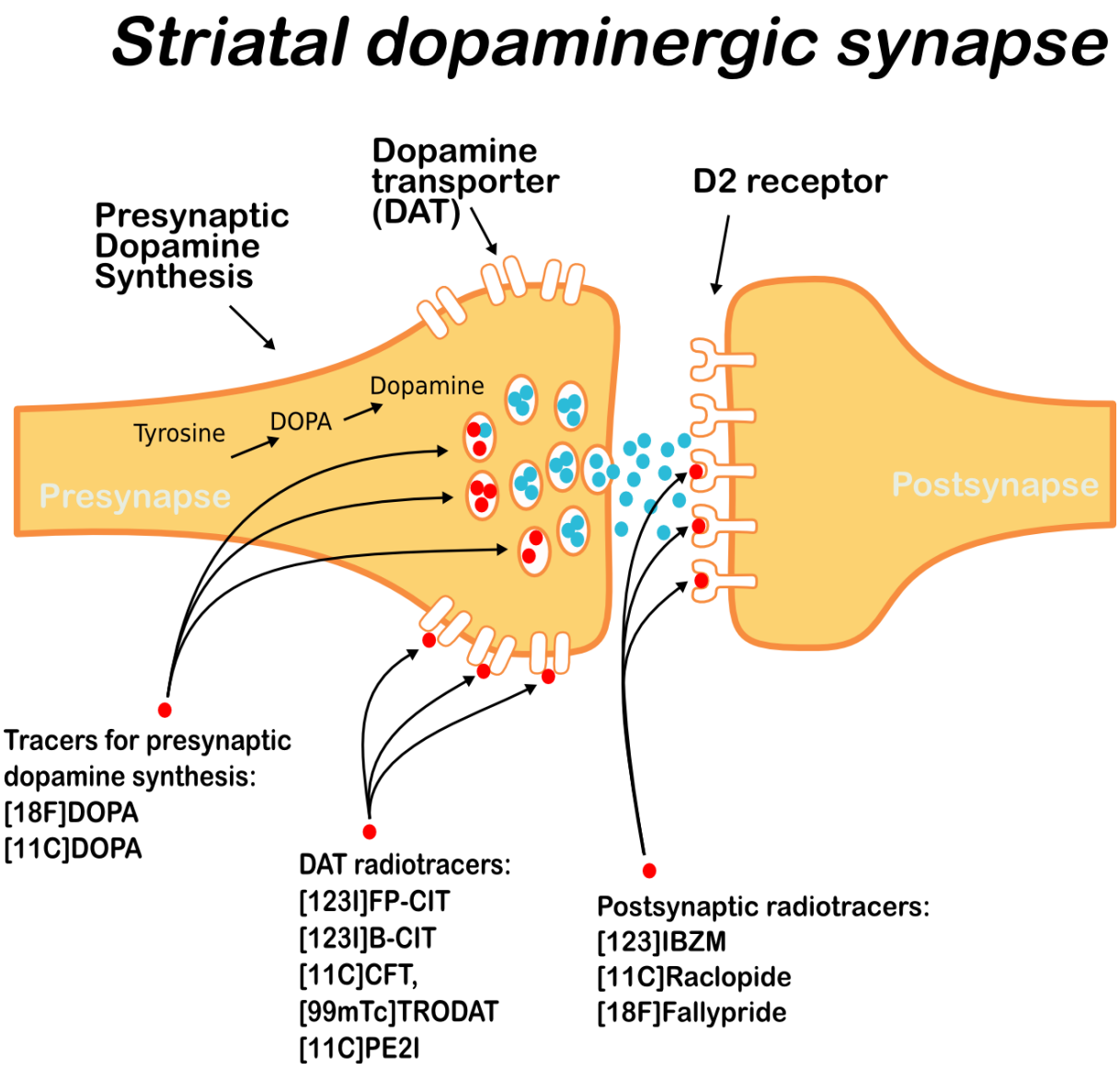

Figure 1: Schematic illustration of a striatal dopaminergic synapse.

Memory impairment in schizophrenia

While positive symptoms often represent the most acute and dramatic changes in patients with schizophrenia, it has been suggested that negative symptoms are most crucial for the long-term outcome of patients[23]. This might be partly because pharmacological treatment can efficiently improve positive symptoms, but has little effect on negative symptoms such as cognitive impairment[24]. Especially memory impairment has been frequently reported in patients with schizophrenia[25,26].

The pathophysiological mechanism underlying these redutions in memory function is not clear. Recent meta-analyses show that patients with schizophrenia exhibit gray matter reductions in brain regions involved in memory processing such as frontal and temporal cortices[27–29]. Particulary the volume of the hippocampus - one of the key regions in memory formation - seems to be reduced in patients with schizophrenia[9,8,7,6]. In the prodromal phase of schizophrenia subjects exhibit attenuated cognitive functioning[30] in a smiliar way. Addionally, these individuals exhibit gray matter alterations[31,32] and these alterations progress during the onset of psychosis[33]. Patients with predominantely negative symptoms such as memory impairment typically exhibit greater gray matter changes then subjects with positive symptoms[34]. Importantly, patients with schizophrenia exhibit not only structural brain changes, but also functional abnormalities in multiple brain regions. Recent meta-analyses of functional neuroimaging studies in patients performing memory tasks support a relationship between cognitive symptoms and abnormal brain function[35–37].

The endophenotype concept in the context of schizophrenia

Since relatives of patients with schizophrenia and especially monozygotic twins have an increased risk of developing schizophrenia, it has been suggested that genetic factors play a role in the pathogenesis. However, large-scale genome-wide association studies have shown heterogenous results and no single gene locus could be indentified so far[38]. To resolve this discrepancy, it has been suggested that genetic effects might be too subtle to be observed on a phenotypic level (e.g. the association between a genetic factor and psychiatric diagnose)[39]. Instead it is recommended to relate genetic effects to an *endophenotype* (e.g. the association between a genetic factor and reduced hippocampal volume). Such biological measures are more proximal to the genetic expression and can potentially be measured more exactly than clinical symptoms[40]. Following this endophenotypic approach, a number of studies have

investigated the influence of potential risk-genes for schizophrenia on brain structure and function[41].

The gene coding for the brain-derived neurotrophic factor (BDNF) is among the most interesting genes in the context of memory functioning. This neurotrophin is expressed in multiple brain areas including the hippocampus[42,43] and takes an important role in structural synaptic changes associated with memory formation[44]. BDNF is synthesized presynaptically as a precursor protein pre-pro-BDNF and subsequently cleaved into pro-BDNF and the mature form of BDNF (see Figure 2). Both pro-BDNF and BDNF are secreted and can induce action at the postsynapse[45(p200)]. The evidence from multiple studies confirms a role of BDNF in memory function and its crucial part in long-term potentiation[45,46].

The gene coding for the protein BDNF is located on chromosome 11. The functional single nucleotide polymorphism (SNP) rs6265 leads to a substitution of a methionine (met) to a valine (val) at codon 66 in the pro-region of BDNF. In a central study by Egan et al.[47] it was shown there is less secretion of BDNF in carriers of the met-variant . Also human subjects that carry the met-variant showed reduced memory performance as compared to val-homozygotes[47]. Several studies investigated the effect of BDNF on hippocampal structure, hippocampal function and memory performance due to its role in memory formation and synaptic plasticity. However, substantial heterogeneity persists regarding the results, methodological details and investigated subjects, providing no clear picture of BDNF's effect on hippocampal structure and function.

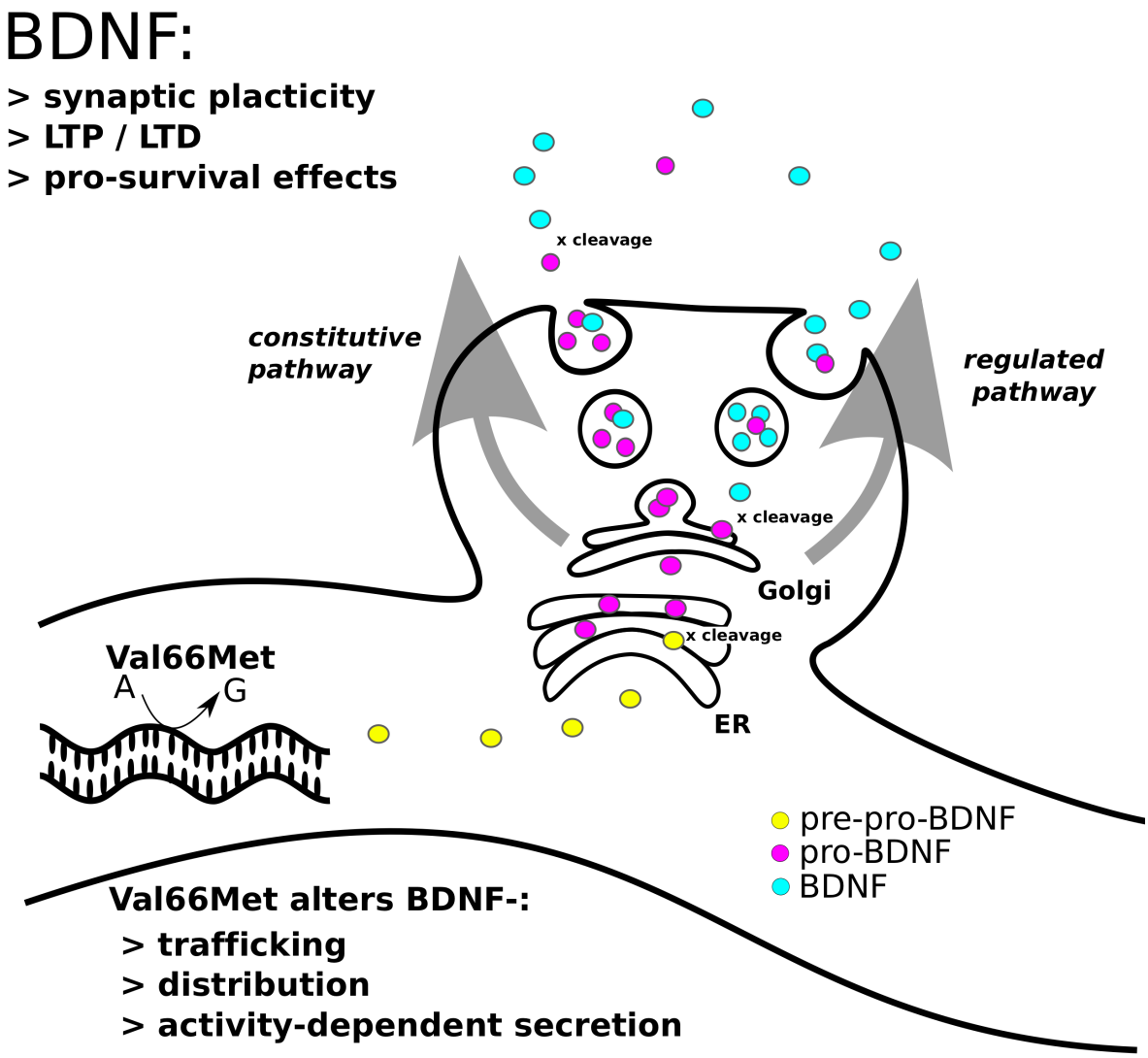

Figure 2: Schematic illustration of the expression and secretion of BDNF

Publications

Publication #1: The nature of dopamine dysfunction in schizophrenia and what this means for treatment

It has been argued that the investigation of the neurobiological underpinnings of the schizophrenia is crucial to develop and provide efficient treatment strategies[3]. The dopamine-hypothesis represents the most popular pathophysiological account of schizophrenia[4,5]. Multiple studies reported changes in striatal dopamine function when applying radiotracers that specifically bind to dopamine receptors, to dopamine transporter or tracers that index presynaptic dopamine synthesis (see [48] for a review). The heterogeneity of the studies with respect to the investigated patient samples and methodological details lead to inconsistent results. Three separate meta-analyses of in-vivo neuroimaging studies have been conducted to investigate the nature of the dopaminergic dysfunction in schizophrenia. The results indicate a significant increase in presynaptic dopaminergic function in patients with schizophrenia (Cohen's d=0.79). There was no evidence for an alteration in dopamine transporter availability and only limited evidence for a small elevation in D(2/3) receptor availability (Cohen d=0.26). There was no significant change in D(2/3) receptor availability if analyses was restricted to drug-naive patients. The presented results suggest that dopaminergic changes are localized presynaptically. Most importantly, current pharmacological treatment which works primarly at the postsynapse, does not affect this elevation. Future research for new pharmacological treatment, should consider targeting presynaptic elevation of dopamine.

Publication #2: Effect of BDNF val(66)met polymorphism on declarative memory and its neural substrate: a meta-analysis

The effects of the met(66)val polymorphism of the BDNF gene was investigated in a comprehensive meta-analysis. In particular we were interested in the effects of this polymorphism on hippocampal structure measured by structural magnetic resonance imaging (MRI) studies and on hippocampal function measured by human memory performance and functional MRI (fMRI) studies. A comprehensive literature search was conducted to indentify all studies suitable for the meta-analyses. Our results indicate small but significant decrease of hippocampal volume in carriers of the met-allele carriers and in line with this finding they showed reduced memory performance across studies. There was a moderate-to-large effect in met-allele carriers showing reduced response of the hippocampus in fMRI studies. These results emphasise the role of BDNF in moderating variability of human memory performance and in mediating some of the neurocognitive impairments underlying neuropsychiatric disorders.

Original articles

ONLINE FIRST

The Nature of Dopamine Dysfunction in Schizophrenia and What This Means for Treatment

Meta-analysis of Imaging Studies

Oliver D. Howes, BM, BCh, MA, MRCPsych, PhD, DM; Joseph Kambeitz, MD; Euitae Kim, MD, PhD; Daniel Stahl, PhD; Mark Slifstein, PhD; Anissa Abi-Dargham, MD; Shitij Kapur, MD, PhD

Context: Current drug treatments for schizophrenia are inadequate for many patients, and despite 5 decades of drug discovery, all of the treatments rely on the same mechanism: dopamine D_2 receptor blockade. Understanding the pathophysiology of the disorder is thus likely to be critical to the rational development of new treatments for schizophrenia.

Objective: To investigate the nature of the dopaminergic dysfunction in schizophrenia using meta-analysis of in vivo studies.

Data Sources: The MEDLINE, EMBASE, and PsycINFO databases were searched for studies from January 1, 1960, to July 1, 2011.

Study Selection: A total of 44 studies were identified that compared 618 patients with schizophrenia with 606 controls, using positron emission tomography or single-photon emission computed tomography to measure in vivo striatal dopaminergic function.

Data Extraction: Demographic, clinical, and imaging variables were extracted from each study, and effect sizes were determined for the measures of dopaminergic function. Studies were grouped into those of presynaptic function and those of dopamine transporter and receptor availability. Sensitivity analyses were conducted to explore the consistency of effects and the effect of clinical and imaging variables.

Data Synthesis: There was a highly significant elevation ($P<.001$) in presynaptic dopaminergic function in schizophrenia with a large effect size (Cohen $d=0.79$). There was no evidence of alterations in dopamine transporter availability. There was a small elevation in $D_{2/3}$ receptor availability (Cohen $d=0.26$), but this was not evident in drug-naive patients and was influenced by the imaging approach used.

Conclusions: The locus of the largest dopaminergic abnormality in schizophrenia is presynaptic, which affects dopamine synthesis capacity, baseline synaptic dopamine levels, and dopamine release. Current drug treatments, which primarily act at $D_{2/3}$ receptors, fail to target these abnormalities. Future drug development should focus on the control of presynaptic dopamine synthesis and release capacity.

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SCHIZOPHRENIA REMAINS ONE OF the leading causes of global disease burden in adults despite more than 50 years of drug development.[1] Understanding its neurobiology is critical for future rational drug discovery.[2,3] The dopamine hypothesis of schizophrenia was first proposed more than 30 years ago on the basis of indirect evidence. It received support from studies of postmortem brain tissue that found increased striatal $D_{2/3}$ receptor density and dopamine levels in patients with schizophrenia and from studies of dopamine and its metabolites in cerebrospinal fluid.[4-8] However, postmortem studies are not able to measure some aspects of the dopaminergic function, such as dopamine release, and are potentially biased by the effects of antipsychotic treatment and agonal events, whereas the cerebrospinal fluid studies were inconsistent and unable to provide insights into the regional aspects of dopamine dysfunction.[9-11] The introduction of positron emission tomographic (PET) and single-photon emission computed tomographic (SPECT) imaging enabled the investigation of in vivo cerebral dopamine neurotransmission free of these limitations.[11-13]

Positron emission tomographic imaging and SPECT imaging have been used to investigate dopaminergic parameters in schizophrenia, beginning with studies of

$D_{2/3}$ receptors[14,15] and later covering presynaptic function, including dopamine synthesis capacity, dopamine release, and transporters[16-19] (see eAppendix [http://www.archgenpsychiatry.com] for further background on these approaches). To our knowledge, there has not been a previous meta-analysis of the presynaptic or dopamine transporter findings in schizophrenia, and since the previous $D_{2/3}$ meta-analysis in drug-free or drug-naive patients,[20] there have been a large number of new studies, which approximately doubles the sample size.

The purpose of our meta-analysis is to synthesize the PET and SPECT imaging findings on dopaminergic function in schizophrenia and to consider their implications for the treatment of schizophrenia. We focus on the striatum because it has the highest density of dopamine projections in the brain[21] and because dopaminergic dysfunction in the striatum can be reliably imaged and has been linked to the severity of symptoms, response to treatment, and the onset of the disorder.[22-25] We group findings into studies of presynaptic dopaminergic function (dopamine synthesis capacity, dopamine release, and synaptic dopamine levels), dopamine transporter availability, and dopamine receptor availability. The studies of dopamine synthesis capacity are grouped with those of dopamine release and synaptic dopamine levels (which use pharmacological challenges that either deplete or release dopamine from presynaptic terminals) because animal[26-28] and in vivo human evidence[29] indicates that they index related aspects of dopaminergic function. However, the results are also given separately for these different methodological approaches for comparison. Researchers can view the study data on, and add future studies to, our open-access database and wiki (http://www.schizophreniadata.com.).

METHODS

DATA SOURCES AND STUDY SELECTION

The PubMed, PsycINFO, and MEDLINE electronic databases were searched in their entirety from January 1, 1960, to July 1, 2011. To be included in the meta-analysis, an article needed to report in vivo PET or SPECT imaging findings on striatal dopaminergic function in patients with schizophrenia and a control group, including the mean and standard deviations for both groups. Current antipsychotic treatment was an exclusion criterion for the studies of dopamine receptors because this affects dopamine receptor binding potential[30] (see eFigure 1 for search results and eAppendix for further details on the search and inclusion-exclusion criteria).

DATA EXTRACTION

The main outcome measure was the difference in the dopaminergic imaging parameter between healthy controls and patients with schizophrenia. The following additional information was extracted from all the studies: authors, year of publication, population characteristics of the control and patient groups (group size, age, sex, antipsychotic use, diagnosis, and symptom ratings), characteristics of the PET or SPECT imaging (radiotracer and other methodological factors reported), scanner characteristics (scanner type and resolution), and modeling method.

DATA ANALYSIS

Separate meta-analyses were conducted for the studies of presynaptic dopaminergic function, dopamine receptors, and dopamine transporters. The standardized effect sizes of the individual studies were entered in a random-effects meta-analytic model.[31,32] The summary effect sizes (Cohen *d*) were computed using a restricted maximum-likelihood estimator.[33] Publication bias was assessed using funnel plots. Heterogeneity was assessed by calculating the I^2 value (I^2 values <50% indicate low to moderate heterogeneity, whereas I^2 values >50% indicate moderate to high heterogeneity).[34] Leave-one-out sensitivity analyses were conducted. Sources of bias and heterogeneity were evaluated using meta-regression (for publication year and age) and subgroup analyses (for antipsychotic treatment, illness duration, and imaging approach). A significance level of $P<.05$ (2-tailed) was used for all analyses (see eAppendix for further methodological details).

RESULTS

PRESYNAPTIC DOPAMINERGIC FUNCTION

A total of 17 studies described in 15 publications (3 studies reported in 1 article[25]) met inclusion criteria. We excluded one of our articles[35] from the main analysis because it reports additional data on the same subjects included in a previous report,[36] although the data are used in subanalyses in which there is no subject duplication, and another article was excluded because the comparator group was siblings.[37] Overall, the studies include a total of 231 patients and 251 controls. Study details are reported in eTables 1 and 2. There was a significant elevation in schizophrenia, with a summary effect size of $d=0.79$ (95% CI, 0.52-1.07; $z=5.65$; $P<.001$; **Figure 1**).

HETEROGENEITY AND SENSITIVITY ANALYSES

The I^2 value was 39.92% (95% CI, 0.00%-77.03%), indicating low to moderate heterogeneity between studies. Although the regression test for funnel plot asymmetry was not significant ($z=1.52$, $P=.13$), a visual inspection of the funnel plot revealed asymmetry, indicating possible publication bias. The trim-and-fill analysis indicated that there were 3 potentially missing studies on the left side of the funnel plot (all with large standard errors and small effect sizes; eFigure 2). Nevertheless, the summary effect size remained large and highly significant after correcting for these putatively missing studies (corrected effect size: $d=0.67$ [95% CI, 0.37-0.94]; $z=4.55$, $P<.001$; $I^2=48.83\%$ [95% CI, 10.17%-81.01%]).

The summary effect size reached significance in all cases in the leave-one-out analysis, with summary effect sizes varying from $d=0.73$ to $d=0.86$ (all $P<.001$). Meta-regression indicated that there was no influence of year of publication ($\beta=-0.02$; $F_{1,13}=0.99$; $P=.34$) or subject age ($\beta=0.004$; $F_{1,12}=0.015$; $P=.90$). In case current antipsychotic drug treatment confounded the results, the meta-analysis was rerun exclusively for studies of drug-free or drug-naive patients. This showed a significant elevation in drug-free or drug-naive patients compared with

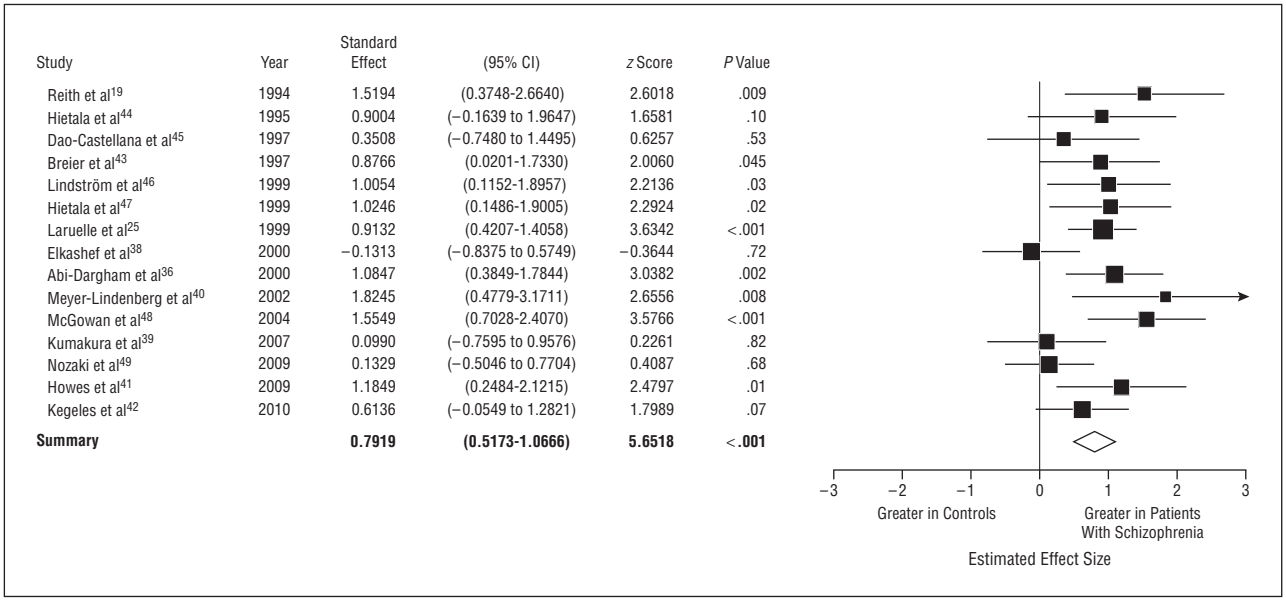

Figure 1. Studies of presynaptic dopaminergic function.[19,25,36,38-49] The forest plot shows the effect sizes and 95% CIs of the difference between patients with schizophrenia and controls, by study. There was evidence of a significant elevation in schizophrenia with a summary effect size of d=0.79 (diamond).

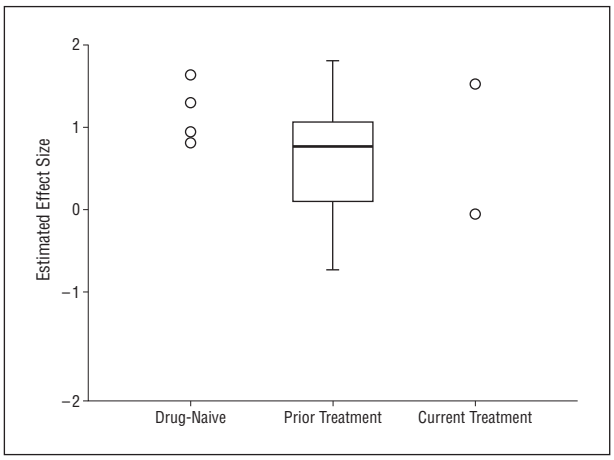

Figure 2. Effect sizes for studies of presynaptic dopaminergic function, by antipsychotic treatment history. In the box plot, the horizontal line represents the median, the whiskers indicate the lowest and highest data points that are within 1.5 the interquartile range, and data outside this range (circles if present) are regarded as potential outliers.

controls (n=13, d=0.69 [95% CI, 0.36-1.01]; z=4.14; P<.001; I^2=46.46% [95% CI, 0.00%-85.31%]). The effect sizes for the studies grouped by antipsychotic treatment are shown in **Figure 2**.

The effect sizes grouped by imaging method are shown in eFigure 3. There was a significant elevation in schizophrenia when the meta-analysis was restricted to the studies using radiolabeled L-3,4-dihydroxyphenylalanine (dopa) (n=11; d=0.78 [95% CI, 0.38-1.18]; z=3.82; P=.0001; I^2=52.62% [95% CI, 3.19%-84.02%]). The effect sizes were similarly positive in the studies of dopamine release (d=1.35 in Abi-Dargham et al,[50] d=0.88 in Breier et al,[43] and d=0.91 in the Laruelle et al[25] report combining 3 cohorts) and in the studies of synaptic dopamine levels (d=1.09 and d=0.61), but there were too few studies to rerun the meta-analysis separately for these approaches.

DOPAMINE TRANSPORTER

Eleven studies met inclusion criteria, providing data on a total of 152 patients and 132 healthy controls. Study details are shown in eTables 3 and 4. There was no evidence of a significant difference between patients with schizophrenia and controls (d=–0.34 [95% CI, –0.75 to 0.07]; z=–1.64; P=.10; **Figure 3**).

HETEROGENEITY AND SENSITIVITY ANALYSES

The I^2 value was 64.04% (95% CI, 25.22%-88.99%), indicating moderate to large heterogeneity between studies. There was no evidence for publication bias (regression test for funnel plot asymmetry: z=–1.75; P=.08; no missing studies estimated by trim-and-fill analysis; see eFigure 4 for the funnel plot) and no significant effect of year of publication (β=–0.01; $F_{1,9}$=0.04; P=.85) or age (β=0.02; $F_{1,9}$=0.25; P=.63) on the effect size. The subgroup analyses found no group differences (eAppendix).

DOPAMINE RECEPTORS

$D_{2/3}$ Receptors

Twenty-two studies met inclusion criteria, providing data on 337 patients and 324 healthy controls (data from Wong et al[15] form part of a subsequent larger data set[62]). The population characteristics and methodological details of the studies are shown in eTables 5 and 6. There was a significant elevation in schizophrenia with a summary effect size of d=0.26 (95% CI, 0.001-0.52; z=1.97; P=.049; **Figure 4**).

Heterogeneity and Sensitivity Analyses

The I^2 value was 63.93% (95% CI, 39.65%-84.81%), indicating moderate to large heterogeneity between stud-

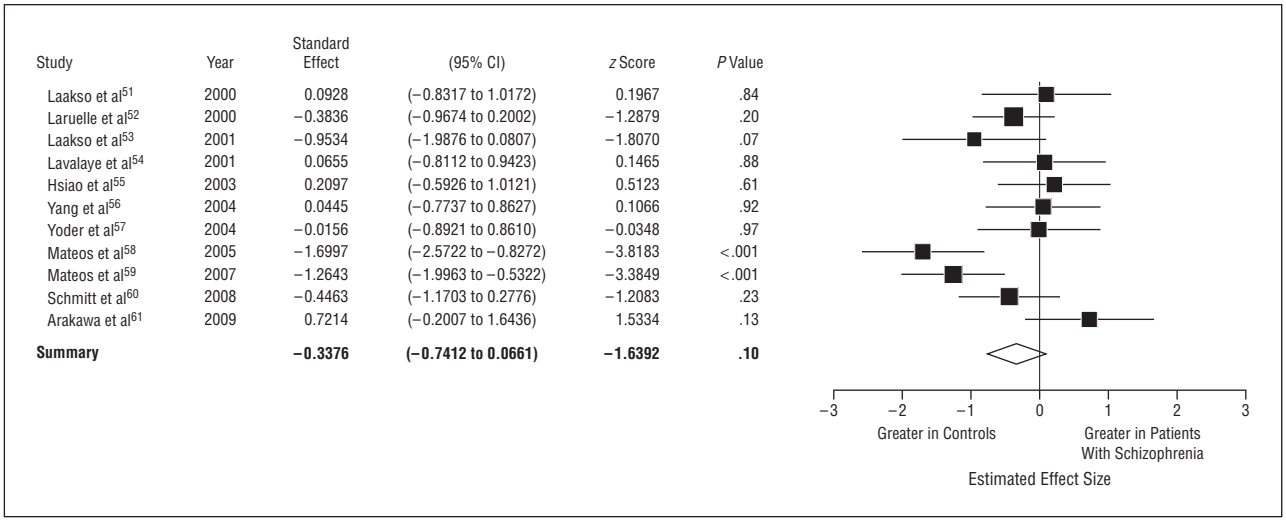

Figure 3. Studies of dopamine transporter availability.[51-61] The forest plot shows the effect sizes and 95% CIs of the difference between patients with schizophrenia and controls, by study. The 95% CI for the summary effect size (diamond; d=0.34) includes 0, indicating no significant difference between patients with schizophrenia and controls.

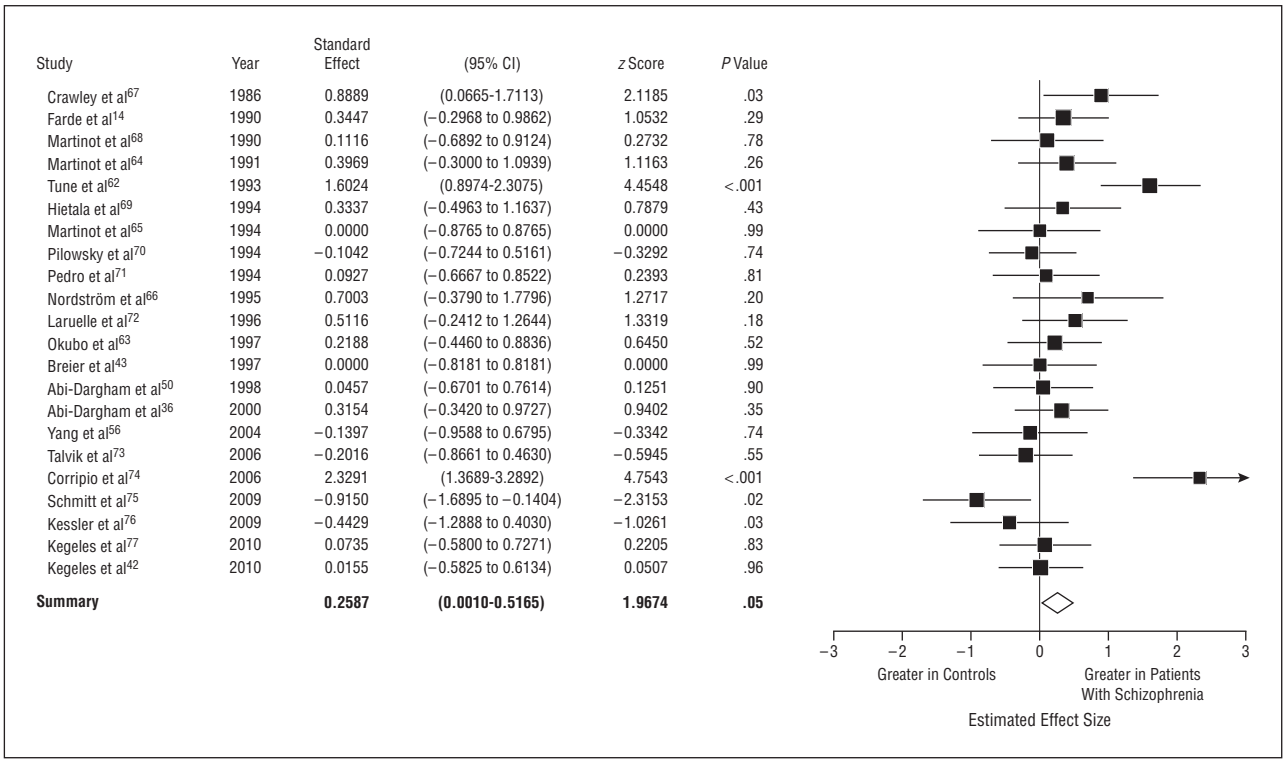

Figure 4. Studies of $D_{2/3}$ receptor availability.[14,36,42,43,50,56,62-77] The forest plot shows the effect sizes and 95% CIs of the difference between patients with schizophrenia and controls, by study. There was evidence of a small increase in D_2 receptor availability in schizophrenia with a summary effect size (diamond) of d=0.26.

ies. There was no evidence for publication bias (regression test for funnel plot asymmetry: z=1.32; P=.19; no missing studies estimated by trim-and-fill analysis; see eFigure 5 for the funnel plot) and no significant effect of year of publication (β=–0.03; $F_{1,19}$=2.27; P=.15) or age (β=0.01; $F_{1,18}$=0.34; P=.57) on the effect size.

In the leave-one-out analysis, the effect sizes varied from d=0.18 to d=0.32 (with P values from .11 to .01, respectively) and were not significant on 14 of the 22 iterations. We repeated the meta-analysis, including a study[78] initially excluded owing to the relatively short antipsychotic drug washout period, and found a nonsignificant effect size of d=0.25 (95% CI, –0.01 to 0.51; z=1.8753; P=.06; I^2=62.75% [95% CI, 38.65%-84.13%]). The subgroup analyses identified no significant difference between patients and controls in studies exclusively of antipsychotic-naive patients or in studies that used benzamide radiotracers, whereas significant differences were found in studies that included patients who had received prior antipsychotic treatment or that used butyrophenone radiotracers (see eAppendix for these analyses and comparisons of illness duration between subgroups).

Other Dopamine Receptors

We identified 4 studies of D_1 receptor availability in untreated patients,[63,79-81] too few to permit meta-analysis. None of these found a significant difference in striatal D_1 availability between patients with schizophrenia and controls, although one study[81] found a trend toward an increase in antipsychotic-naive patients but not drug-free patients (see eAppendix for overview).

STRIATAL SUBREGIONS

We repeated the meta-analyses for the caudate and putamen separately. In the studies of presynaptic function, there was a significant elevation in schizophrenia for the putamen (see eAppendix for details: $d=0.51$ [95% CI, 0.14-0.88]; $z=2.72$; $P=.007$) but not the caudate. There were no significant differences in the caudate or putamen between patients and controls in the studies of dopamine transporter or $D_{2/3}$ receptor availability (see eAppendix for details).

COMMENT

The main findings from our meta-analyses are that presynaptic dopaminergic function is altered in schizophrenia, with a large effect size ($d=0.79$), and that there is no difference in dopamine transporter availability and a small elevation in $D_{2/3}$ receptor availability, although the latter finding was not consistent. These findings are summarized schematically in **Figure 5**.

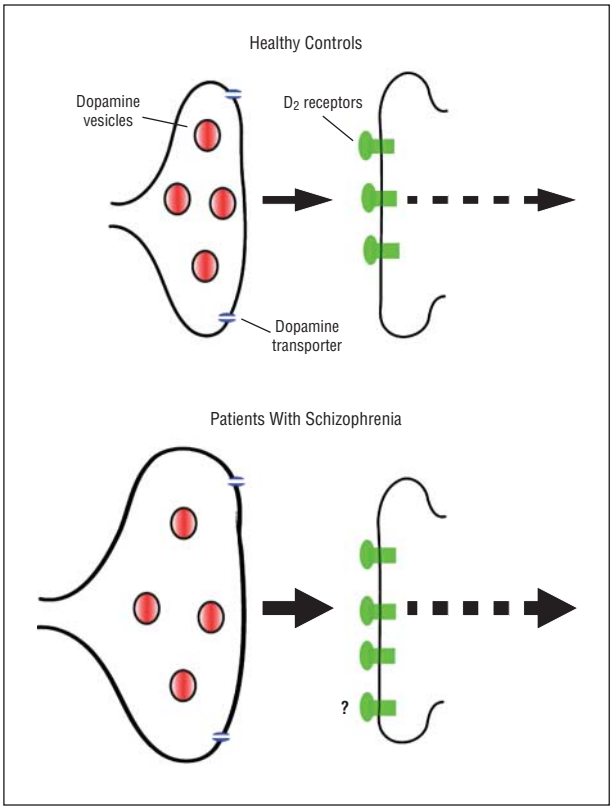

Figure 5. Schematic diagram summarizing the findings from our meta-analyses of dopamine function in schizophrenia. The diagram shows that the major dopaminergic abnormality in schizophrenia is presynaptic. The main findings from our meta-analyses are that presynaptic dopaminergic function is altered in schizophrenia, with a large effect size ($d=0.79$), and that there is no difference in dopamine transporter availability and a small elevation in $D_{2/3}$ receptor availability, although the latter finding was not consistent.

METHODOLOGICAL CONSIDERATIONS

One methodological consideration common to all meta-analyses is that they are limited by the quality of the studies that are included. We included all relevant studies and did not apply quality screening because this may introduce other biases, although this involves pooling findings from studies using different radiotracers, scanners, and methods of data collection and pharmacokinetic analysis. We have summarized these variables (eTables 1-6) to enable readers to make judgments about individual studies. Although including all studies has the advantage of reducing selection biases and increasing the generalizability of findings, there is a risk of diluting effects.

There was low to moderate heterogeneity in the studies of presynaptic dopaminergic function, which suggests that there is consistency across studies. However, there was moderate to large heterogeneity in the studies of dopamine transporter and $D_{2/3}$ receptor availability. Potential sources for this were evaluated in secondary analyses and are discussed herein. Nevertheless, because the random-effects model used in the meta-analyses does not assume homogeneity of effects, our findings should be robust to heterogeneity.

Presynaptic Dopaminergic Function

Although the trim-and-fill analysis indicated that there may be missing studies, the elevation in patients remained large and highly significant after correcting for putatively missing studies. There was a highly significant and large effect size in all the iterations of the leave-one-out analysis, which indicates that the elevation in presynaptic dopaminergic function was not dependent on the inclusion of any one study. We found a large positive effect size when the meta-analysis was restricted to studies that used radiolabeled dopa to index dopamine synthesis capacity, and although there were insufficient studies to permit separate meta-analyses, there were similar positive effect sizes in the studies that used α-methylparatyrosine or amphetamine challenges, which suggests that the elevation is consistent across technique. The elevation was evident when studies of patients currently receiving antipsychotic treatment were excluded from the meta-analysis, which indicates that antipsychotic treatment is unlikely to explain the effect. We cannot, however, exclude the possibility that prior treatment had a persistent effect in the studies of drug-free patients, although Figure 2 indicates that, in absolute terms, the effect sizes were at least as great in the studies of drug-naive patients as in the studies of patients who had received prior treatment, which suggests that this is not the case.

The radiolabeled dopa studies used several different analytic and imaging methods, including the simple ra-

tio approach that does not account for many of the complexities of radiolabeled dopa analysis and is highly dependent on scanning duration,[82] factors that may contribute to the negative effect size in the only study to use this approach.[38] Nevertheless, that the elevation in schizophrenia was evident across studies using a variety of methods and analytic approaches suggests it is robust.

The elevation in presynaptic dopaminergic function could be due to an increased density of dopamine terminals in the striatum. However, this interpretation is unlikely for 2 reasons: first, there is no evidence of a similar elevation in dopamine transporter availability in our meta-analysis or in the vesicular monoamine transporter (both in vivo markers of dopamine neuron terminal density),[83,84] and, second, dopamine neuron numbers are not elevated in postmortem samples.[85] Thus, this indicates that the increased dopamine synthesis capacity and dopamine release reflect functional changes rather than increased neuronal density. Although elevated dopamine synthesis capacity could reflect increased enzyme activity in compensation for reduced dopa or dopamine levels, this interpretation is not consistent with the evidence that synaptic dopamine levels and dopamine release, respectively, are also increased and positively correlated.[35] Together, the presynaptic studies thus suggest that there is increased dopaminergic activity reflected in increased dopamine synthesis capacity and increased dopamine release.[35] This is consistent with evidence of increased turnover of striatal dopamine in schizophrenia.[39] Further work is needed to determine whether dopamine synthesis capacity is related to dopamine release in schizophrenia, as has been found for synaptic dopamine and dopamine release,[35] and whether other aspects of dopaminergic function (eg, conversion of tyrosine to dopa, and dopamine catabolism) are also abnormal.

Dopamine Transporter Availability

There was no evidence of publication bias. Antipsychotic treatment is unlikely to explain our finding because most of the patients in the dopamine transporter studies were drug-naive, and the lack of difference between patients and controls was also evident when the studies of treated patients were excluded. A likely source of the heterogeneity between studies is the number of different radiotracer imaging approaches used, although we were not able to formally assess this. Differences in clinical characteristics, such as variation in the severity and phase of illness and in drug-free intervals, are evident between studies (eTables 3 and 4) and may be a further source of heterogeneity between studies.

Dopamine Receptor Availability

There was no evidence of publication bias. There was no significant difference between patients and controls on 14 of the 22 iterations of the leave-one-out analysis, which indicates that the finding of a difference in the meta-analysis is not robust. In the sensitivity analyses, we could not detect a difference between patients and controls when the meta-analysis was restricted to purely drug-naive patients or when it was restricted to patients who had received prior treatment scanned with benzamide radiotracers. The 2 studies[64,65] that used ergot radiotracers included a mixture of drug-naive and previously treated patients and found no difference between patients and controls, in line with the findings with benzamide radiotracers. However, when the meta-analysis was restricted to butyrophenone radiotracers, there was an elevation in patients. Interestingly, this was not evident in the one butyrophenone study[66] exclusively of drug-naive patients. These further analyses thus suggest that the imaging approach used and the inclusion of patients who had received prior antipsychotic treatment are likely to contribute to the inconsistency in the meta-analysis. Other differences in clinical characteristics may also contribute to this inconsistency: in particular, duration of illness (which was shorter in the drug-naive patients), whether illness duration included the prodrome, and the nature and severity of symptoms (eTable 6).

There are differences in the pharmacokinetic properties of the different radiotracers and in the analytic methods used to characterize them and their pharmacodynamics,[86-88] so it is not possible to disentangle which of these factors might underlie the effect of imaging approach on our findings. For example, in comparison with the benzamide radiotracer raclopride, in membrane, slice, and cell preparations, the butyrophenone radiotracers *N*-methylspiperone and spiperone have shown paradoxical binding decreases following dopamine depletion[89,90] and either increases or no overall change following stimulated release.[89-91] Some studies,[91] although not all,[92] have found that spiperone has a greater tendency to bind to internalized receptors than does raclopride. *N*-methylspiperone and spiperone also have a higher affinity for $D_{2/3}$ receptors than does raclopride (K_d values for *N*-methylspiperone and spiperone are in the picomolar range and, those for raclopride are in the nanomolar range), and they have slower kinetics,[86] which makes it more difficult to obtain quantitative estimates from short-duration PET studies and necessitates the use of a different kinetic model for analysis.[15,93]

When evaluating the sensitivity analyses, it is also important to consider that the risk of type II errors increases when the number of studies is reduced, and there is an inevitable decrease in the precision of the estimate. This is reflected in the wide confidence intervals for the drug-naive and drug-free groupings, and therefore the finding of a lack of a significant difference in the drug-naive studies needs to be seen in the context of the reduced power to find such a difference. Finally, elevated baseline synaptic dopamine in schizophrenia could potentially make group differences harder to detect. Nevertheless, overall, one can conclude that, although there was a small elevation in $D_{2/3}$ receptor availability, it was not a consistent finding and was not present in drug-naive patients, although some caveats remain.

IMPLICATIONS FOR THE DOPAMINE HYPOTHESIS OF SCHIZOPHRENIA

Our findings provide in vivo evidence to support the dopamine hypothesis of schizophrenia. Early versions of this hypothesis could only conjecture the nature of the ab-

normality.[94] This meta-analysis provides evidence to specify that the major dopaminergic abnormality in schizophrenia is a presynaptic one, affecting dopamine synthesis capacity and release, and that, in contrast, the overall effect on $D_{2/3}$ receptor availability is small. This view is supported by findings of elevated dopamine synthesis capacity in drug-naive individuals in the prodrome to schizophrenia[24] and of a further increase associated with the onset of the psychotic disorder.[95] There is also evidence of specificity because this presynaptic dopaminergic dysfunction is not seen in nonpsychotic affective and anxiety disorders (see review by Howes et al[16]). Although we were unable to examine symptoms in our meta-analyses, the challenge studies[17,36] link elevated dopamine release to positive rather than negative symptoms.

Although our findings support proposals that dopaminergic dysfunction is a final common pathway to psychosis, they do not address the issue of what drives the presynaptic striatal alterations. One candidate is decreased D_1-mediated dopaminergic neurotransmission in the frontal cortex (see Fusar-Poli et al[96] and Meyer-Lindenberg et al[40] and review by Heinz et al[97]). Another candidate, supported by preclinical models and some human findings,[98-100] is glutamatergic dysfunction.

Our finding that dopamine transporter availability is unaltered indicates that there is no elevation in transporter levels that might compensate for elevated dopamine release. It may also explain the later age of onset of schizophrenia in women than men, because women tend to have higher dopamine transporter availability than men, which naturally declines with age in both sexes.[101] Although our findings indicate that transporter availability is unaltered, it remains possible that transporter *function* is altered in schizophrenia.

Because we focused on the striatum, it is not possible to know whether our presynaptic findings are specific to the striatum or whether they are also relevant to dopaminergic projections to other brain regions, and future work will need to evaluate the extrastriatal dopamine system. Our analyses of striatal subregions suggest that the presynaptic elevation may be localized to the putamen. However, these findings should be considered as exploratory because not all studies provided data and because the resolution of scanners varied markedly (eTable 1). The putamen localization contrasts with recent findings focusing on functional, as opposed to purely anatomical, subregions of the striatum, which have suggested that the dopaminergic dysfunction is localized in a part of the caudate nucleus that is linked to associative cortical regions.[41,42] Unfortunately, there were too few studies for the functional subregions to be examined in our meta-analysis, and therefore studies using high-resolution scanners are warranted to examine subregional effects further.

IMPLICATIONS FOR TREATING SCHIZOPHRENIA

The current drug treatments for schizophrenia were discovered prior to the notions of dopamine as a neurotransmitter and prior to our ability to measure its function in vivo in humans. They were the outcome of empiricism and serendipity, rather than rational drug design based on pathophysiology. It has transpired that the major mode of action of all currently licensed antipsychotic drugs is to block D_2 receptors.[9,102] However, our meta-analysis indicates that, by blocking D_2 receptors, current drugs are acting downstream of the locus of the largest dopaminergic abnormality in the disorder. Thus, although antipsychotics suppress overall neurotransmission, they fail to target the major dopaminergic abnormality. Furthermore, our finding that the $D_{2/3}$ alterations were not present in drug-naive patients suggests that $D_{2/3}$ receptor alterations are not intrinsic to the illness but are secondary to prior antipsychotic treatment. Although studies are needed to test this after accounting for the factors already discussed, this interpretation is consistent with animal evidence that antipsychotics result in $D_{2/3}$ receptor upregulation[103] and with evidence that withdrawing antipsychotic drugs in humans uncovers elevated $D_{2/3}$ receptor availability.[104] It is not surprising that when antipsychotics are stopped (usually by the patient), when there is nothing to suppress the dysregulated presynaptic dopaminergic system, and when there is a potentially supersensitive postsynaptic receptor system, then there is a high risk of relapse.

Our findings indicate that, rather than focusing exclusively on postsynaptic receptors, future treatments should target the presynaptic control of dopamine synthesis and release. Interestingly, one of the first effective drug treatments for schizophrenia was reserpine,[105] and more recent data show that use of α-methylparatyrosine is associated with a rapid and profound reduction in psychotic symptoms.[36] Because both of these drugs deplete the store of presynaptic dopamine, there is thus proof of principle that, by acting on the presynaptic dopaminergic system, we can treat the psychosis. However, although presynaptic dopamine depletion seems logical from a pathophysiological perspective, it raises a technical challenge because dopamine and norepinephrine share part of the same synthetic pathway. Thus, treatments that interfere with dopamine also risk affecting norepinephrine synthesis, leading to undesirable adverse effects. Therefore, future efforts at presynaptic modulation will need to go beyond the simple depletion of dopamine or blockade of its synthesis because the cost-benefit ratio of this is unlikely to be therapeutically viable. Future efforts will also probably need to show some regional selectivity if they are to avoid altering dopamine neurotransmission in the frontal cortex and potentially worsening negative symptoms and cognitive impairments, both of which have been linked to frontal cortical D_1 receptor availability in schizophrenia.[63]

Interestingly, patients who respond less well to antipsychotic drugs have been found to show lower synaptic dopamine levels,[36] and findings indicate that treatment-resistant patients show normal dopamine synthesis capacity.[106] These findings suggest that psychotic symptoms in some patients may be unrelated to dopaminergic function, at least as indexed by these imaging techniques.

Although we did not find a major alteration in dopamine transporter or $D_{2/3}$ receptor availability, there could nevertheless be other functional alterations. In fact, this is indirectly suggested by findings that patients with

schizophrenia are supersensitive to the psychotogenic effects of the D_2 receptor agonist apomorphine when given at high doses.[107] Interestingly, when apomorphine is given at low doses, which are thought to have a preferential presynaptic action to reduce dopaminergic transmission, it has an antipsychotic effect.[108] D_2 receptors may exist in forms with differing affinities for dopamine, and it has been proposed that there is an excess of the high-affinity form in schizophrenia.[107] However, the first in vivo study[109] in schizophrenia using a radiotracer selective for the high-affinity form found no evidence of alterations, although a significant caveat is that this radiotracer also shows appreciable binding to D_3 receptors. Notwithstanding this, other aspects of $D_{2/3}$ receptor function (such as internalization or signal transduction) or the function of other dopamine receptors could be abnormal in schizophrenia and warrant investigation in patients. If these or other aspects of D_2 function are abnormal, this would suggest new drug targets, and even if D_2 function is unaltered, finding new ways to intervene at this level could still be useful to counteract the effects of presynaptic dysfunction on dopamine neurotransmission.

An attractive feature of the present findings is that the pathophysiological target (ie, increased dopamine synthesis capacity and dopamine release) can now be measured in preclinical models and humans using exactly the same molecular imaging techniques as has been done for dopamine transporters and $D_{2/3}$ receptors.[110] So, although most of the animal models used to develop antipsychotics in the past have had to rely on indirect measures (such as amphetamine-induced locomotion or conditioned avoidance response abolition), the present findings provide a pathophysiological target that can be directly measured in animals. With advances in small animal imaging and experimental human studies, it should be possible to induce the precise presynaptic abnormality in animal models and to measure the response to new medications in animals and in experimental human models in the same way.

In conclusion, there is consistent evidence of presynaptic dysfunction in schizophrenia with a large effect size but no evidence of a compensatory increase in dopamine transporter availability to buffer the system. $D_{2/3}$ receptor upregulation is small and not detected in antipsychotic-naive patients. These findings suggest that drug development should target the presynaptic regulation of dopamine synthesis and release.

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Supplementary Online Content

Howes OD, Kambeitz J, Kim E, Stahl D, Slifstein M, Abi-Dargham A, Kapur S. The nature of dopamine dysfunction in schizophrenia and what this means for treatment: Meta-analysis of imaging studies. *Arch Gen Psychiatry*. Published online April 2, 2012. doi:10.1001/archgenpsychiatry.2012.169.

eAppendix. Supplementary material

eFigure 1. Flowchart showing how the papers were identified for inclusion

eFigure 2. Studies of presynaptic dopaminergic function (funnel plot)

eFigure 3. Studies of presynaptic dopaminergic function (box plot)

eFigure 4. Studies of the dopamine transporter (funnel plot)

eFigure 5. Studies of D2 receptor availability (funnel plot)

eFigure 6. Studies of D2 receptor availability: box plots of effect sizes, by antipsychotic treatment history

eFigure 7. Studies of D2 receptor availability: box plots of effect sizes, by class of radiotracer used

eReferences

eTable 1. Methodological characteristics of the studies of presynaptic dopaminergic function

eTable 2. Subject characteristics of the studies of presynaptic dopaminergic function

eTable 3. Methodological characteristics of the studies of dopamine transporter availability

eTable 4. Subject characteristics of the studies of dopamine transporter availability

eTable 5. Methodological characteristics of the studies of dopamine receptor availability

eTable 6. Subject characteristics of the studies of dopamine receptor availability

This supplementary material has been provided by the authors to give readers additional information about their work.

eAppendix

Supplementary Background

A number of PET and SPECT imaging techniques have been used to study *in vivo* dopaminergic function in schizophrenia. Dopamine synthesis capacity can be indexed using two radiolabeled homologues of *l*-3,4-dihydroxy-phenylalanine (DOPA): [β-^{11}C]L-DOPA ([^{11}C]-DOPA) and 6-[^{18}F]fluoro-DOPA ([^{18}F]-DOPA).[1, 2] Brain metabolism of radiolabeled-DOPA parallels that of endogenous L-DOPA.[3] In dopamine neurons, these radiotracers are converted by aromatic L-amino acid decarboxylase (AADC) into [^{11}C]dopamine and 6-[^{18}F]fluoro-dopamine, respectively, and trapped in vesicles in the nerve terminals ready for release (see review[1]). AADC is a regulated enzyme and its activity in dopamine neurons is relative to other aspects of dopamine metabolism.[4] AADC is present in other monoaminergic neurons in addition to dopamine neurons.[5] Nevertheless, radiolabeled-DOPA uptake in the striatum is predominantly due to dopaminergic innervation, is highly correlated with striatal dopamine levels in post mortem brains, and responds to experimental manipulation of brain dopaminergic systems.[5-8]

The next stage of dopaminergic transmission is dopamine release into the synapse. Synaptic levels of dopamine can be indexed by imaging the effect of competition between dopamine and radiotracers which selectively bind to dopamine D2/3 receptors, such as [^{11}C]-raclopride, and [^{123}I]-iodobenzamide, on the availability of these receptors.[9] The competition model indicates that radiotracer binding will decrease when synaptic dopamine levels are increased, for example with dopamine release after amphetamine administration, and conversely that binding will increase when synaptic dopamine levels are reduced, for example after depletion of presynaptic dopamine stores achieved with alpha-*methyl*-paratyrosine (AMPT) administration (see reviews [9, 10]). Supporting this, *in vivo* animal studies show that specific binding by the radiotracer decreases monotonically with increasing dopamine levels measured by microdialysis.[11] Studies have shown that the competition model alone does not account for all of the observations yielded by these imaging paradigms, and that receptor trafficking likely plays a role, but nevertheless, changes in radiotracer binding are related to the overall net effects of these events, which are a direct consequence of the change in dopamine tone produced by pharmacological or other challenges.[12, 13]

Following its release, dopamine diffuses across the synapse to act on post-synaptic dopamine receptors. A large number of radiotracers have been developed to image D2 receptors, including benzamides (including [^{11}C]-raclopride, [^{18}F]-fallypride, [^{11}C]-FLB457 and [^{123}I]-iodobenzamides), ergot derivatives (including [^{76}Br]-bromolisuride) and the butyrophenones (including [^{18}F]-spiperone, [^{11}C]-spiperone, [^{76}Br]-bromospiperone, and [^{11}C]-NMSP).[9, 10] These do not distinguish D2 from D3 receptors or pre- from post-synaptic receptors and vary somewhat in their properties, including selectivity for D2/3 receptors over D4 receptors and kinetics (see[10] and discussion). Selective tracers are also available for D1 receptors and are being developed for D4 and D5 receptors.

Subcortical dopaminergic neurotransmission is predominantly terminated by dopamine diffusion out of the synapse and reuptake into the nerve terminal by dopamine transporters. Dopamine

transporters can be imaged using PET or SPECT radiotracers such as [^{123}I]-β-CIT, TRODAT, [11C]-cocaine, [11C]-methylphenidate, [18F]CFT ([18F]-WIN 35,428) and [^{11}C]-PE2I.[14]

Supplementary Methods

The following keywords were used in the database searches: “Positron Emission Tomography”, OR “PET”, OR “Single photon emission tomography”, OR “SPET”, OR “Single Photon Emission Computed Tomography” OR “SPECT”; AND “dopamine”, OR “dopamine release”, or “dopamine synthesis”, or “dopamine availability”, OR “dopamine transporter”, OR “dopamine reuptake”, OR “dopamine receptor”; AND “schizophrenia”, OR “psychosis”, OR “schizophreniform”.

The inclusion criteria were: peer-reviewed studies that reported an *in vivo* measure of striatal dopaminergic function in patients with a diagnosis of schizophrenia and in a healthy control group. We excluded case studies, reviews, studies of patients with co-morbid neurological diagnoses, and duplicate publications. The abstracts of all papers identified by the search were screened by OH, EK & JK to determine if they met inclusion criteria. If the abstract indicated the study potentially met inclusion criteria, or where there was any uncertainty, the full text of the paper was reviewed to identify studies that met all the inclusion criteria and to ensure they did not have any of the exclusion criteria. Where there was uncertainty, authors were contacted to confirm that no overlap in the studied participants existed between papers. Current antipsychotic treatment was an exclusion criterion for the studies of dopamine receptors, because this affects dopamine receptor binding potential.[15] Where antipsychotic treatment was stopped prior to scanning we looked for evidence that there was a sufficient wash-out period (at least 5 times longer than the half-life of the antipsychotic drug in plasma) such that residual antipsychotic occupancy of D2/3 receptors was unlikely.

Meta-analytic Procedure

The statistical analysis of the extracted data was conducted using the R statistical programming language version 2.10.1 with the packages ‘rmeta’ and ‘metafor’.[16] Most studies reported data for the whole striatum. However, some only reported data for striatal sub-regions (caudate nucleus and putamen) without reporting values for the whole striatum. In order to achieve higher comparability between studies where data for the whole striatum was not available, an effect size for the whole striatum was calculated.

For studies where data for the whole striatum was not available, the effect size for the whole striatum was calculated by averaging the means of the dopaminergic index in putamen and caudate nucleus weighted by their volume to reflect the relatively larger contribution of the putamen to the overall striatal volume. Where volumes were not reported, the following volumes, derived from healthy controls (n=34, mean age=32.5 (SD=8.8) years), were used: mean (SD) volume (mm^3): putamen=8805 (994), caudate=5562 (865)). When estimating the standard deviation of this striatal measure, we accounted for the dependency of measures in striatal subregions by assuming a correlation of r = 0.5 between measures in striatal sub-regions.

We investigated the validity of this approach by using two studies included in the meta-analysis where data were available for the putamen and caudate and for the whole striatum.[17, 18] We evaluated the intra-class correlation coefficient (ICC) for the whole striatal values determined by combining data from the caudate and putamen as described above and the values for the whole striatum reported in these studies using a mixed effects two-way ANOVA.[19] The ICC was high

(ICC=+0.98, F(24,24)=94.3, p<0.0001, 95%-CI for ICC: 0.95 to 0.99), indicating that our approach to combining regions gives an accurate estimate of the whole striatal values.

The standardized effect sizes of the individual studies were entered in a random-effects meta-analytic model [20, 21], which does not assume homogeneity amongst studies. The summary effect sizes (Cohen's *d)* were computed using a restricted maximum-likelihood estimator.[22] Heterogeneity was assessed in the studies by calculating the I^2 value, which is a sample size independent measure that describes the percentage of total variation across studies that is due to heterogeneity rather than chance.[23] I^2 values of 25%, 50%, and 75% can be interpreted as indicating low, moderate and high heterogeneity respectively.[23]

Where there was a significant difference between patients and controls in the meta-analysis, a sensitivity analysis was conducted using the leave-one-out approach, which re-runs the meta-analysis repeatedly with a different study excluded on successive iterations.

We evaluated potential sources of heterogeneity in the effect sizes and the influence of possible confounding factors in the following ways. The potential effects of publication year and the age of subjects was evaluated using meta-regression.[20] Additionally, to investigate the influence of antipsychotic treatment, where there were ≥5 studies in a group, we re-ran the meta-analyses separately for studies grouped by antipsychotic treatment (drug-naïve/ drug-free or currently receiving drug treatment). Where there were <5 studies in a group we plotted the individual effect sizes but did not enter them into a meta-analysis because this becomes unreliable with a small numbers of studies. We used the same approach to investigate whether the different radiotracer imaging methods used contributed to heterogeneity (see Supplementary Tables 1-6 for the groupings).

Publication bias was evaluated by inspection of the funnel plot for evidence of asymmetry. A funnel plot is a plot of each study's effect size on the x-axis against its standard error (1/precision). Publication bias is suggested if trials in the left hand corner (small precision and small effect size) are omitted, creating a degree of asymmetry in the funnel plot. Publication bias was further evaluated using a regression test for funnel plot asymmetry, and the trim-and-fill analysis, which provides an estimate of the meta-analysis if there has been publication bias.[24, 25]

Supplementary Results

Dopamine transporter- sub-group analyses

We repeated the meta-analysis including only studies of patients who were drug-naive (6 studies). This showed no significant difference between patients and controls (*d*=-0.44; 95%-CI: -0.99 to 0.12, z=-1.54, p=0.12, I^2=69.28%, 95%-CI for I^2: 25.48 to 93.81%). There was no significant difference in illness duration between drug-naive subjects (mean (sd) =13 (11) months) and subjects taking antipsychotic drugs (mean (sd) = 150 (43) months; t=4.44, df=1.04 (corrected for unequal variances), p=0.13). There were too few studies of patients currently taking neuroleptics (n=4) or of drug-free patients (n=1) to enable separate meta-analysis and insufficient studies in each group to enable a separate meta-analysis for the different radiotracer imaging approaches used.

Dopamine receptor- sub-group analyses

Studies were grouped into those of patients who had never received antipsychotic treatment (drug naive) and those including patients who had received previous antipsychotic drug treatment (prior treatment). The antipsychotic-naïve group (n=6 studies) showed no significant difference between patients and controls (see Supplementary Figure 6, *d*=0.27, 95%-CI: -0.57 to 1.11, z=0.62, p=0.53, I^2=86.08%, 95%-CI of I^2: 63.02 to 97.88 %). However, the prior treatment group (n=15 studies) showed evidence of an elevation in D2/3 receptors in patients (*d*=0.28, 95%-CI: 0.03 to 0.52, z=2.20, p=0.03, I^2=40.96%, 95%-CI of I^2: 0 to 76.44%). The duration of illness was significantly longer in the prior treatment patients (mean (sd)=124 (63) months) than in drug-naive patients (mean (sd)= 15 (4) months, t = -4.21, df=5.1 (corrected for unequal variances), p=0.008).

The effect sizes for the different radiotracer imaging methods used are shown in Supplementary Figure 7. The meta-analysis was re-run separately for the studies that used a benzamide and for those that used a butyrophenone radiotracer (there were too few studies to enable this for those using ergot derivative radiotracers). There was no significant difference between patients and controls in the studies using a benzamide radiotracer (n=14; *d*=0.13, 95%-CI: -0.19 to 0.44, z=0.78, p=0.44, I^2=63.26%, 95%-CI of I^2: 63.26 to 89.40%). There was, however, a significant elevation in the patients in the studies that used a butyrophenone radiotracer (n=5; *d*=0.71, 95%-CI: 0.14 to 1.28, z=2.44, p=0.01, I^2=60.85%, 95%-CI of I^2: 0 to 94.52 %). There was no significant difference in duration of illness between studies using benzamides (mean (sd) = 75 (79) months) and those using butyrophenones radiotracers (mean (sd) = 129 (44) months, t=1.23, df=3.2 (corrected for unequal variances), p=0.3).

Given that most of the studies that used butyrophenone radiotracers included patients who had received prior antipsychotic treatment, prior treatment and radiotracer used were potentially confounded. To explore this we excluded the studies that had used butyrophenones, and repeated the meta-analysis of the remaining studies of patients who had received prior antipsychotic treatment (n=11). This found no significant difference between patients and controls (*d*=0.09, 95%-CI: -0.13 to 0.32, z=0.81, p=0.41; Supplementary Figure 6), and low heterogeneity (I^2=0%, 95%-CI of I^2: 0 to 42.11 %).

Dopamine D1 receptors

We identified six studies that measured striatal D1 receptor availability in patients with schizophrenia, although two studies included patients who were taking antipsychotic drugs at the time of scanning.[26-31] Three of the studies, comprising 43 patients in total (23 antipsychotic free and 20 antipsychotic naive), found no difference in striatal D1 availability[27-29], whilst two, comprising 15 patients in total (all taking antipsychotic drugs) found a reduction[26, 30], and one found a trend-level increase in antipsychotic-naïve patients (n=12) that was not present in previously treated but drug free patients (n=13). Although the investigators in the two studies that found a reduction selected patients who were taking antipsychotic drugs with relatively low affinity for D1 receptors, antipsychotic occupancy of D1 receptors cannot be excluded and could explain the reduction in these two studies. There were too few studies of drug free patients to enable a meta-analysis of striatal D1 availability in schizophrenia.

Meta-analysis for striatal sub-regions

To evaluate whether our analyses for the whole striatum were obscuring important sub-regional differences, we repeated the meta-analysis for striatal sub-regions where sufficient data were available (a minimum of five studies). There were sufficient studies to enable this for the caudate and putamen, but not for the nucleus accumbens or for functional sub-divisions of the striatum.

Pre-synaptic dopaminergic function studies

Caudate:

Eight studies provided data for the caudate. The meta-analysis of these studies found no significant difference between patients and controls (d=0.37, z=1.57, p=0.12, 95%-CI: -0.09-0.82, I2=54.04%, 95%-CI of I2: 0-90.74%).

Putamen:

Eight studies provided data for the putamen. The meta-analysis of these studies found a significant elevation in schizophrenia, with an effect size of *d*=0.51 (z=2.71, p=0.007, 95%-CI: 0.14-0.88, I2=29.98%, 95%-CI of I2: 0-80.07%).

Dopamine transporter studies

Caudate:

Eight studies provided data for the caudate. The meta-analysis of these studies found no significant difference between patients and controls (*d*=-0.43, z=-1.60, p=0.11, 95%-CI: -0.95 to -0.09, I2=65.09%, 95%-CI of I2: 20.25 to -91.48%).

Putamen:

Eight studies provided data for the putamen. The meta-analysis of these studies found no significant difference between patients and controls (*d*=-0.4, z=-1.41, p=0.16, 95%-CI: -0.95 to -0.15, I2=68.97%, 95%-CI of I2: 28.83-92.51%).

Dopamine D2/3 receptor availability

Caudate:

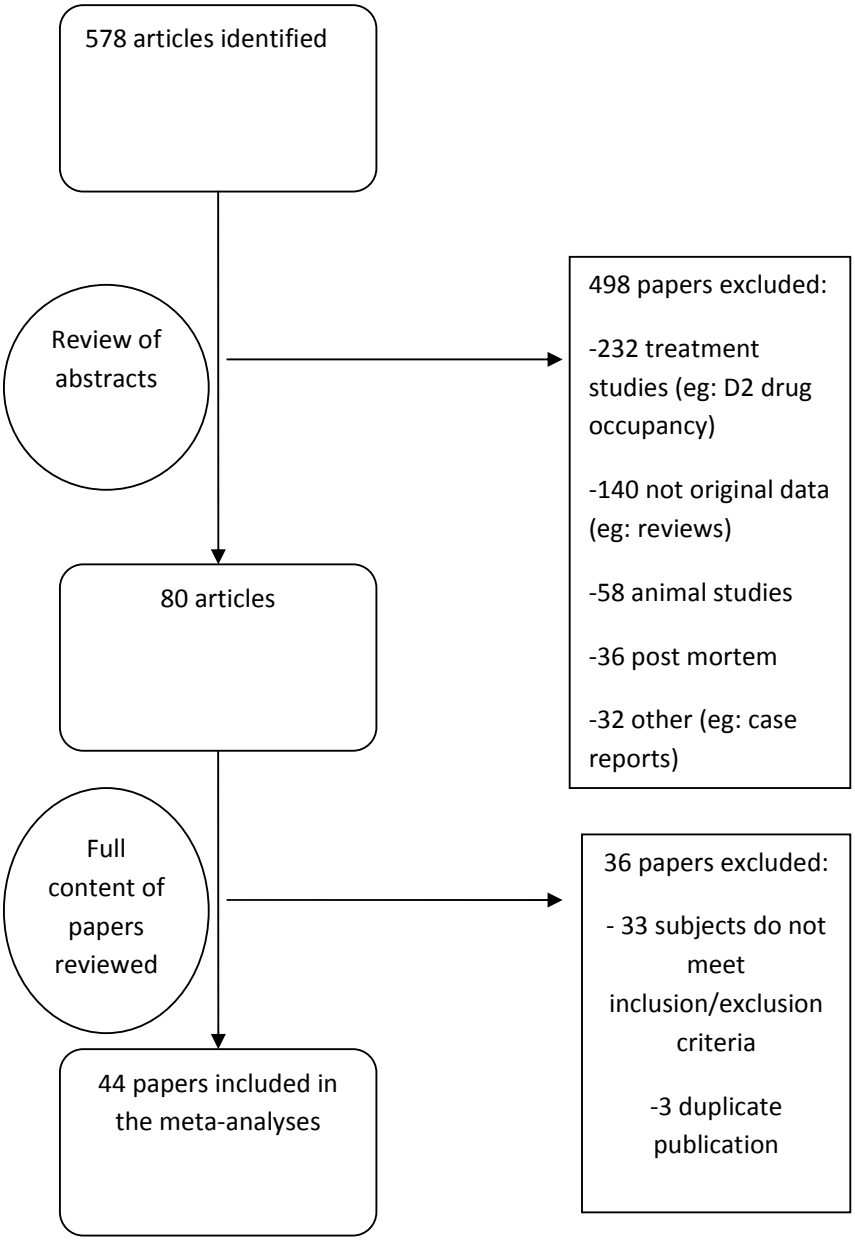
Five studies provided data for the caudate. The meta-analysis of these studies found no significant difference between patients and controls (*d*=0.32, z=0.96, p=0.33, 95%-CI: -0.33 to -0.97, I2=78%, 95%-CI of I2: 36.98-97.48%).

Putamen:

Five studies provided data for the putamen. The meta-analysis of these studies found no significant difference between patients and controls (*d*=0.02, z=0.13, p=0.9, 95%-CI: -0.3 to -0.34, I2=0%, 95%-CI of I2: 0-90.88%).

Supplementary Figures

eFigure 1: Flowchart showing how the papers were identified for inclusion



*Notes:

Presynaptic dopaminergic function: one paper[32] combined data from two previous studies[33, 34] with new data from an additional study. As this is the most complete data set, we used this report in the main meta-analysis but include data from the other studies in sub-analyses where there is no overlap in subjects, and for this reason these papers are also reported in the Tables. One study was

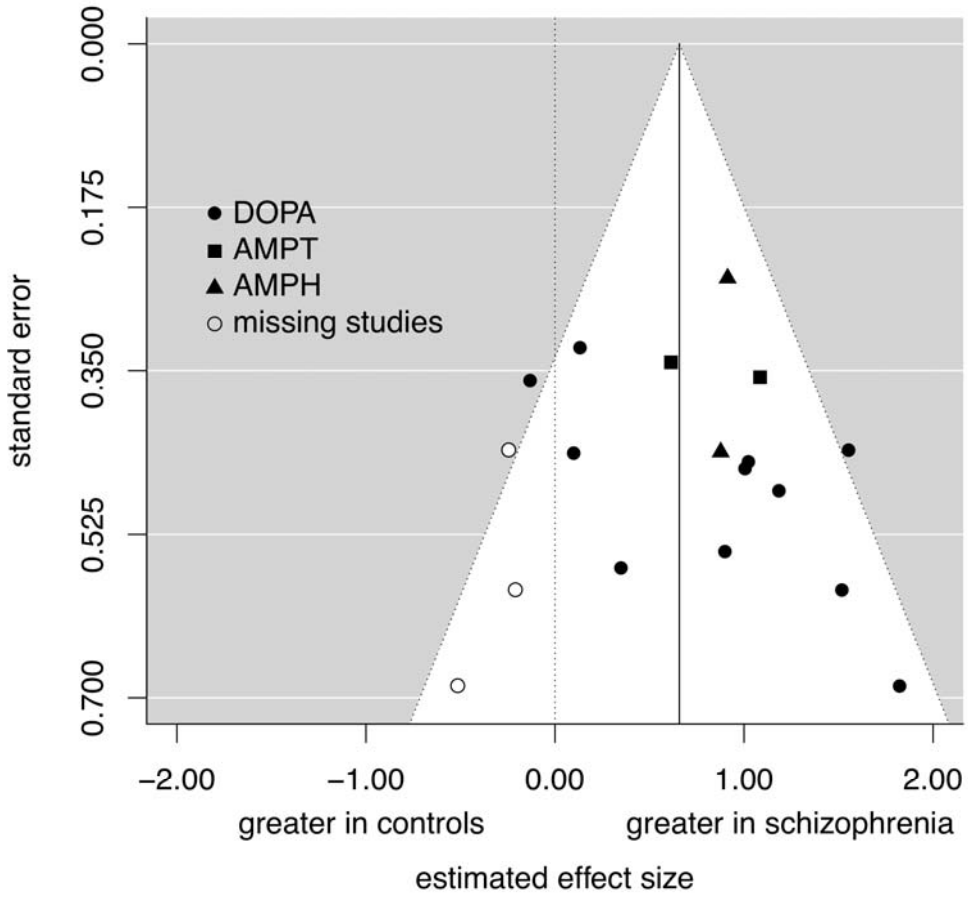
of patients with schizophrenia and their well siblings. This study was excluded because the comparator group was related to the patients.[35]

Dopamine transporter availability: One study was excluded because the patients had a co-morbid neurological disorder associated with dopamine neuron loss (parkinson's disease) in addition to schizophrenia[36], and another was excluded as data were only reported as percentage of control values without reporting actual values.[37]

Dopamine receptor availability: one paper[38] combined data from one previous study[39] with additional new data and was used as it is the most complete data set. One study included subjects who were scanned 7 days after stopping antipsychotic treatment, and was excluded from the main analysis because of the risk of residual antipsychotic occupancy of D2/3 receptors (see above), but was included in a further sensitivity analysis.[40]

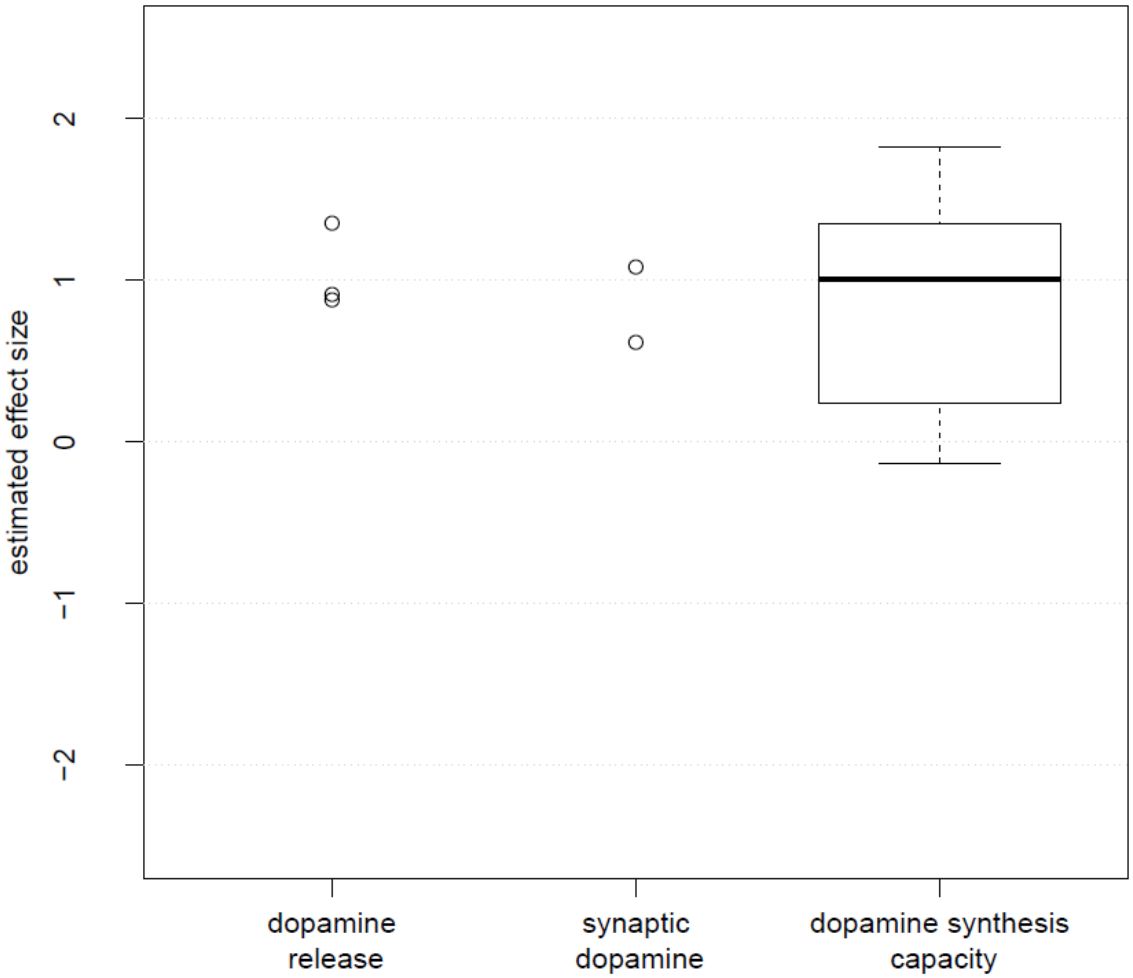
eFigure 2

Studies of presynaptic dopaminergic function: Funnel plot showing the effect sizes for each study (studies using radiolabeled DOPA [filled circles]; alphamethyl-*para*-tyrosine [AMPT; filled squares] to index synaptic dopamine levels; amphetamine [AMPH; filled triangles] to index dopamine release) and potentially missing studies (open circles) based on the trim-and-fill analysis, which assumes the effect sizes follow a normal distribution.



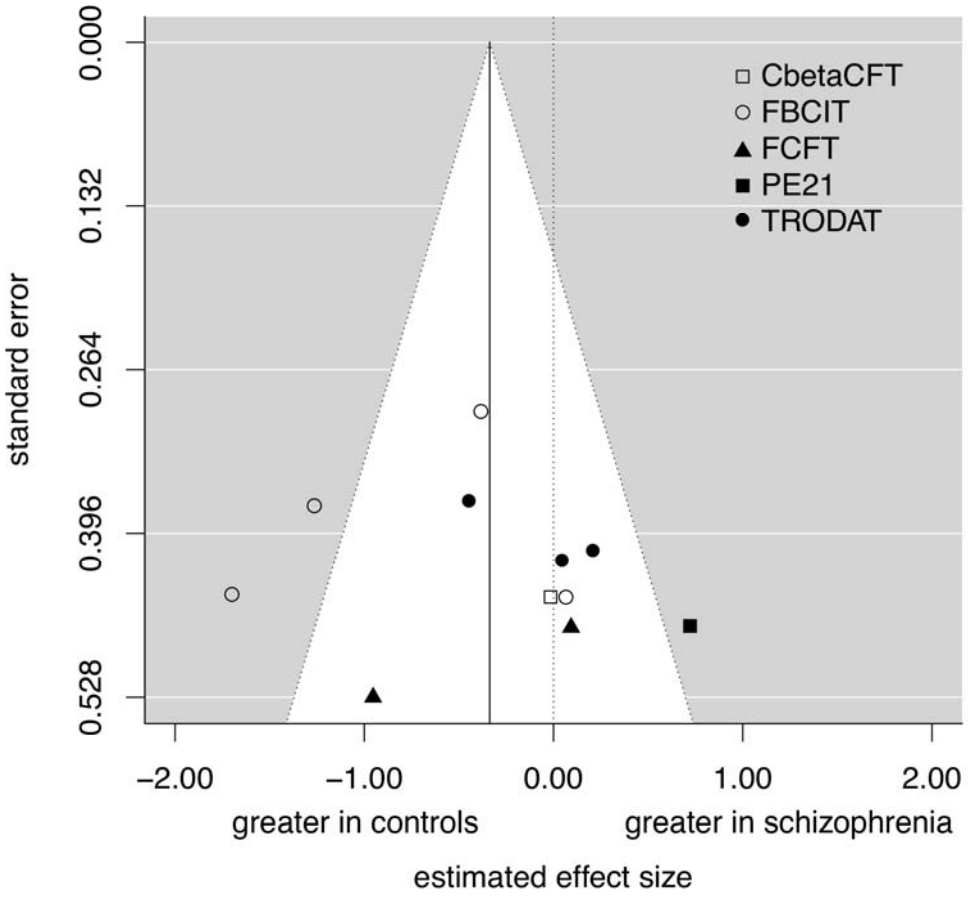
eFigure 3

Studies of presynaptic dopaminergic function: Showing the effect sizes by imaging approach used in the studies. Where n>4 studies in a group, the effect sizes are summarised using a boxplot (in the boxplot in the boxplot the band is the median and the whiskers indicate the lowest and highest data points that are within 1.5 * the inter-quartile range, and data outside this range (circles if present) are regarded as potential outliers), otherwise the effect size for each study is plotted). Studies using change in radiotracer binding following AMPT or amphetamine are grouped as 'synaptic dopamine' and 'dopamine release' respectively (effect sizes shown for Laruelle et al 1999, which combines 3 dopamine release studies and is used in the main meta-analysis as it is the most complete data-set; Breier et al, 1997; and Abi-Dargham et al 2009, although this latter study is not included in the main meta-analysis because the subjects also took part in the Abi-Dargham et al 2000 AMPT study). Studies using radiolabeled DOPA radiotracers are in the 'dopamine synthesis capacity' group



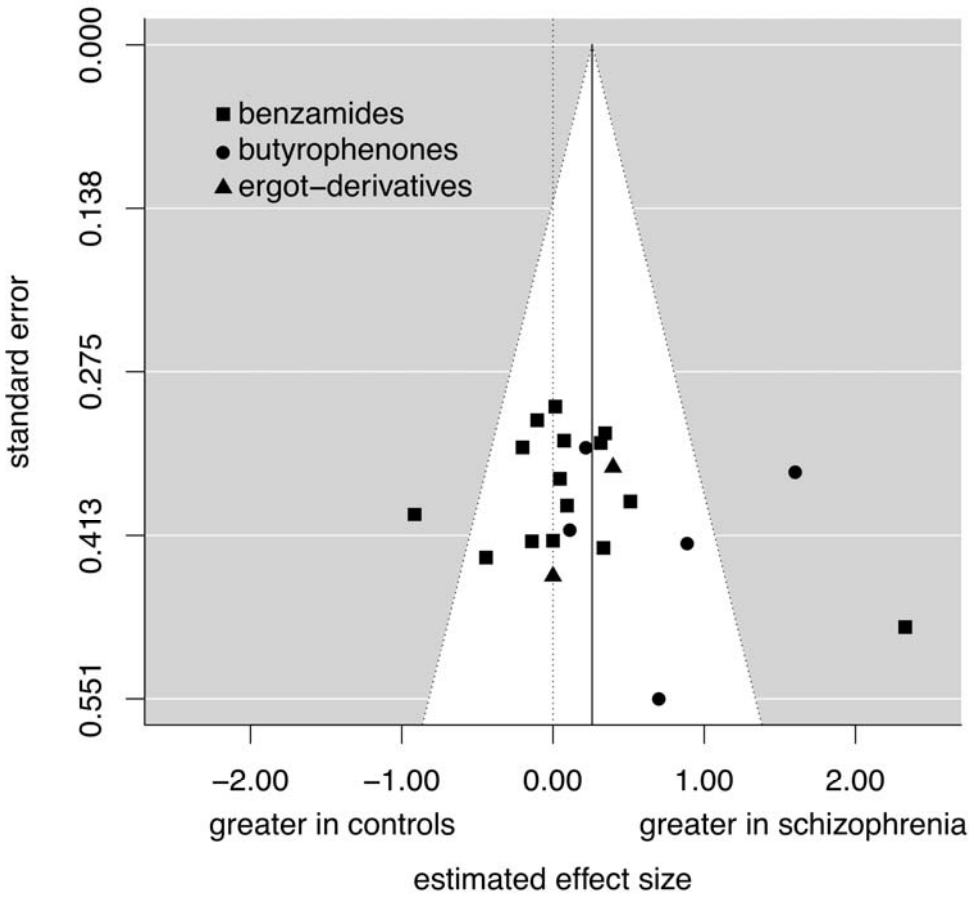
eFigure 4

Studies of the dopamine transporter: Funnel plot showing the effect sizes for each study (studies using TRODAT [filled circles]; PE2I [filled square]; FCFT [filled triangle];FBCIT [open circles]; CbetaCFT [open square]).



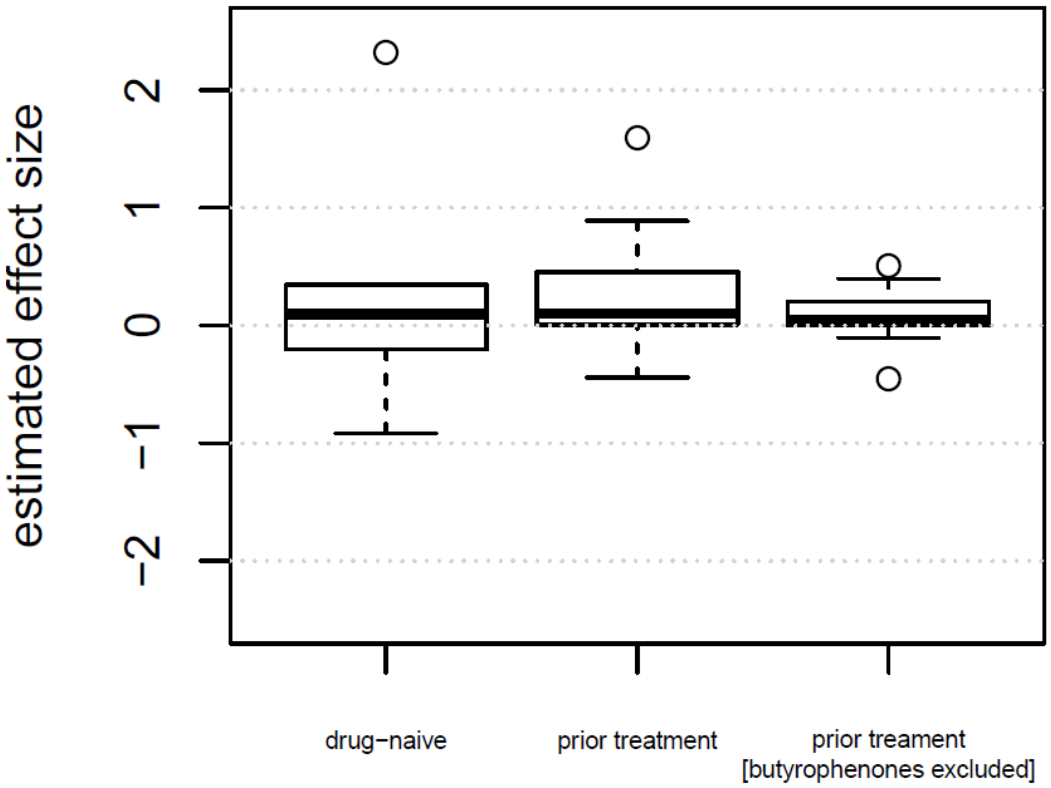
eFigure 5

Studies of D2 receptor availability: Funnel plot showing the effect sizes for each study



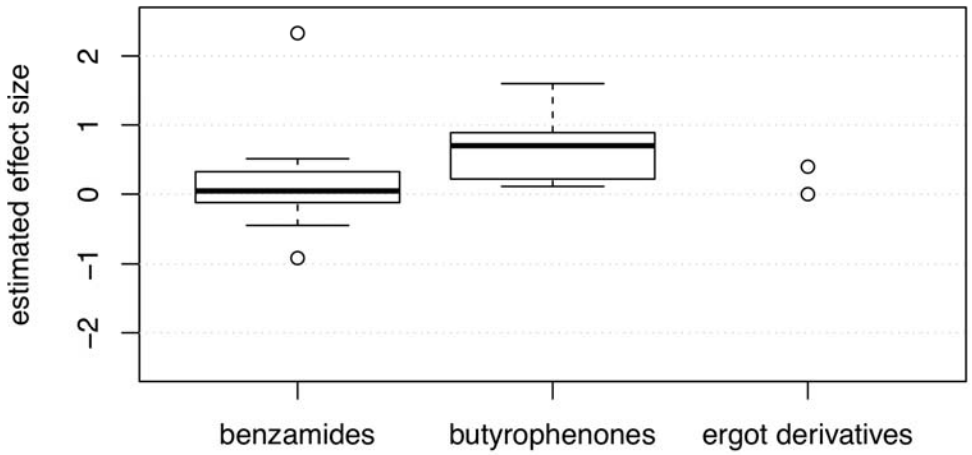
eFigure 6

Studies of D2 receptor availability: Boxplots of the effect sizes for studies by antipsychotic treatment history (in the boxplots the band is the median and the whiskers indicate the lowest and highest data points that are within 1.5 * the inter-quartile range, and data outside this range (circles if present) are regarded as potential outliers).



eFigure 7

Studies of D2 receptor availability: Effect sizes by class of radiotracer used in the studies. Where n>4 studies in a group the effect sizes are summarised using a boxplot (the band is the median and the whiskers indicate the lowest and highest data points that are within 1.5 * the inter-quartile range, and data outside this range (circles if present) are regarded as potential outliers)



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eTable 1. Methodological characteristics of the studies of presynaptic dopaminergic function

	Author	PET Tracer	Imaging approach	Radio-tracer delivery	Drugs administered prior to scanning	Scanner Type	Resolution (FWHM mm)	Outcome Measure	Reference region
Radiolabelled DOPA studies	Reith et al 1994[1]	[^{18}F]Fluoro-L-DOPA	single scan	bolus	-	PC-2048B; Scanditronix	na	k_3	cortex
	Hietala et al 1995[2]	[^{18}F]Fluoro-L-DOPA	single scan	bolus	-	ECAT 931/08-12	na	k_i	occipital cortex
	Dao-Castellana et al 1997[3]	[^{18}F]Fluoro-L-DOPA	single scan	bolus	-	ECAT-Siemens 953-B	6.26	k_i	occipital cortex
	Hietala et al 1999[4]	[^{18}F]Fluoro-L-DOPA	single scan	bolus	Carbidopa: 100 mg, 1.5h pre-scanning	ECAT 931/08-12	na	k_i	occipital cortex
	Lindstroem et al 1999[5]	[^{11}C]DOPA	single scan	bolus	-	GEMS PC2048-15B	5	k_i	occipital cortex
	Elkashef et al 2000[6]	[^{18}F]Fluoro-L-DOPA	single scan	bolus	Carbidopa: 150 mg amino acid infusion	2048-15B; Scanditronix	6.5	uptake ratio (striatum/ ref)	occipital cortex
	Meyer-Lindenberg et al 2002[7]	[^{18}F]Fluoro-L-DOPA	single scan	bolus	Carbidopa: 100 mg	PC-2048-153; Scanditronix	6.5	k_i	occipital cortex
	McGowan et al 2004[8]	[^{18}F]Fluoro-L-DOPA	single scan	bolus	Carbidopa: 150 mg, Entacapone: 400 mg	HR++/966 EXACT; CTI PET Systems	4.8	k_i	occipital cortex
	Kumakura et al 2007[9]	[^{18}F]Fluoro-L-DOPA	single scan	bolus	Carbidopa: 2mg/kg, 1h pre-scanning	ECAT EXACT 47, Siemens	na	k_{in}^{app}	cerebellum
	Nozaki et al 2009[10]	[^{11}C]DOPA	single scan	bolus	-	ECAT/EXACT HR; CTI-Siemens	7.5	k_i	occipital cortex
	Howes et al 2009[11]	[^{18}F]Fluoro-L-DOPA	single scan	bolus	Carbidopa: 150 mg, Entacapone: 400 mg	HR++/966 EXACT; CTI PET Systems	4.8	k_i	cerebellum
Dopamine release (amphetamine) studies	Laruelle et al 1996[12]	[^{123}I]IBZM	two scan (baseline and active)	bolus+ infusion	active scan: 0.3 mg/kg amphetamine IV bolus	PRISM 3000 Picker	11	ΔBP	occipital cortex
	Breier et al 1997[13]	[^{11}C]Raclopride	two scan (baseline and active)	bolus+ infusion	active scan: 0.2 mg/kg amphetamine	General Electric Advance	6	ΔBP	cerebellum
	Abi-Dargham et al 1998[14]	[^{123}I]IBZM	two scan (baseline and active)	bolus+ infusion	active scan: 0.3 mg/kg amphetamine IV bolus	PRISM 3000 Picker	11	ΔBP	occipital cortex
	Laruelle et al 1999[15]	[^{123}I]IBZM	two scan (baseline and active)	bolus+ infusion	active scan: 0.3 mg/kg amphetamine IV bolus	PRISM 3000 Picker	11	ΔBP	occipital cortex
	Abi-Dargham et al 2009[16]	[^{123}I]IBZM	two scan (baseline and active)	bolus+ infusion	active scan: 0.3 mg/kg amphetamine IV bolus	na	na	ΔBP	average of frontal and occipital cortex
Synaptic dopamine (AMPT) studies	Abi-Dargham et al 2000[17]	[^{123}I]IBZM	two scan (baseline and active)	bolus+ infusion	active scan: 8g AMPT[1] PO over 2 days	PRISM 3000 Picker	11	ΔBP	average of frontal and occipital cortex
	Kegeles et al 2010[18]	[^{11}C]Raclopride	two scan (baseline and active)	bolus+ infusion	active scan: 12.9-16.9 mg/kg AMPT[1] PO over 2 days	ECAT/EXACT HR; CTI-Siemens	4.4/ 4.1	ΔBP	cerebellum

[1]alpha-methyl-*para*-tyrosine; $K_{3\ (K3^D)}$=relative activity of dopa decarboxylase, K_i=utilization rate constant of DOPA relative to a reference region; K_{in}^{app}=net blood-brain DOPA clearance, BP=binding potential, FWHM=full width half maximum

eTable 2. Subject characteristics of the studies of presynaptic dopaminergic function

	Authors	Controls		Patients									
		N (m/f)	Age mean (sd)/yr	N (m/f)	Age mean (sd)/yr	Diag-nosis [1]	Inclusion criteria for diagnosis	Exclusion criteria	Illness duration	Antipsychotic treatment	Total symptom score (mean [sd])	Positive symptom score (mean [sd])	Negative symptom score (mean [sd])
Radiolabelled DOPA studies	Reith et al 1994	13 (9/4)	36 (13)	5 (5/0)	38 (4)	All SZ	DSM-III-R	na	14 years	4 naïve, 1 free for >3 years	PANSS: 58 (na)	PANSS: 14 (3)	PANSS: 12 (2)
	Hietala et al 1995	8 (6/2)	27 (7)	7 (4/3)	26 (7)	All SZ	DSM-III-R	na	24 months	all drug naïve	PANSS: 81 (14)	na	na
	Dao-Castellana et al 1997	7 (na)	25 (5)	6 (na)	26 (9)	All SZ	DSM-III-R	neurological/ severe somatic disorders, alcoholism, toxicomania	6 years	2 naïve, 4 free for ≥4 months	PANSS: 94 (na)	PANSS: 21 (12)	PANSS: 33 (7)
	Hietala et al 1999	13 (8/5)	30.4 (9.4)	10 (4/6)	29.6 (8.8)	7 SZ, 3 SZD	DSM-III-R	na	7 months	All naïve	PANSS: 77.6 (na)	na	na
	Lindstroem et al 1999	10 (8/2)	na	12 (10/2)	31 (na)	All SZ	DSM-III-R	abnormality on CT, EEG or routine blood tests, positive urine drug screen	31.08 months	10 naïve, 2 drug free for >2 years	na	na	na
	Elkashef et al 2000	13 (8/5)	34.6 (10.75)	19 (15/4)	36.3 (na)	All SZ	DSM-III-R	medical/ neurological disorders, alcohol or drug abuse	17.3 years	10 taking drugs, 9 drug free	na	na	na
	Meyer-Lindenberg et al 2002	6 (5/1)	34 (na)	6 (5/1)	35 (na)	All SZ	DSM-III-R	na	na	all free for ≥6 weeks	na	na	na
	McGowan et al 2004	12 (12/0)	38.3 (7.1)	16 (16/0)	39.9 (11.3)	All SZ	DSM-IV	neurologic/serious physical illness, substance abuse	na	All on long-term drug treatment	CASH: 10.6 (na)	CASH: 4.2 (na)	CASH: 6.3 (na)
	Kumakura et al 2007	15 (15/0)	37.3 (6.4)	8 (8/0)	37.3 (6.3)	All SZ	DSM-IV	psychoactive medication	na	3 naive, 6 free for ≥6 months	PANSS: 80.2 (4.7)	PANSS: 15.4 (3.5)	PANSS: 23.6 (4.0)
	Nozaki et al 2009	20 (10/ 10)	35.1 (9.5)	18 (10/8)	35.6 (7.4)	All SZ	DSM-IV	brain disease, substance abuse, or episode of mood disorder	26.4 months	14 naïve, 4 free	PANSS: 79.2 (21.4)	PANSS: 22.6 (7.3)	PANSS: 17.1 (6.5)
	Howes et al 2009	12 (8/4)	24.3 (4.6)	7 (5/2)	36.0 (14.7)	All SZ	DSM-IV	neurologic/ medical illness, head injury, alcohol or drug abuse or dependence	na	2 naive, 5 free for >8 weeks	PANSS: 61.7 (31.0)	PANSS: 17.0 (7.0)	PANSS: 16.1 (10.0)

Dopamine release (amphetamine) studies	Laruelle et al 1996	15 (14/1)	41 (2)	15 (14/1)	42 (2)	All SZ	DSM-IV	other DSM-IV axis I diagnosis, substance abuse or dependence, severe medical condition	14 years	all free (mean free period=192 days)	BPRS: 37 (3)	PANSS: 16.1 (1.7)	PANSS: 14.9 (1.5)
	Breier et al 1997	12 (9/3)	29.2 (9.01)	11 (8/3)	32.4 (9.95)	All SZ	DSM-IV	illegal drug dependence and/or significant drug abuse, severe head trauma, significant medical condition	6.6 years	4 naive, 7 free for >14 days	BPRS: 28.8 (7.2)	BPRS: 6.7 (2.8)	na
	Abi-Dargham et al 1998	15 (12/3)	40 (11)	15 (12/3)	41 (9)	All SZ	DSM-IV	other DSM-IV axis I diagnosis, substance abuse or dependence, severe medical conditions	17 years (2 FE)	2 naive, 13 free	BPRS: 44 (11)	PANSS: 18.5 (5.1)	PANSS: 19.6 (7.0)
	Laruelle et al 1999	36 (32/4)	40 (9)	34 (28/6)	40 (9)	All SZ	DSM-IV	other DSM-IV axis I diagnosis, substance abuse or dependence, severe medical conditions	na	7 naive, 27 free for 104 days (mean)	na	PANSS: 17.5 (6.2)	PANSS: 16.8 (6.6)
	Abi-Dargham et al 2009	8 (6/2)	28 (8)	6 (4/2)	28 (8)	All SZ	DSM-IV	Na	FE	all drug naive	na	na	na
Synaptic dopamine (AMPT) studies	Abi-Dargham et al 2000	18 (11/7)	31 (8)	18 (11/7)	31 (8)	All SZ	DSM-IV	other DSM-IV axis I diagnosis, substance abuse or dependence, severe medical conditions	na	8 naïve, 10 free for 139 days (mean)	PANSS: FE: 71 (12) Chronic: 63 (11)	PANSS: 18.2 (6)	PANSS: 13.8 (5.4)
	Kegeles et al 2010	18 (13/5)	29 (7)	18 (13/5)	29 (8)	All SZ	DSM-IV	weight <50kg or> 115kg, other DSM-IV axis I diagnosis, substance abuse or dependence, severe medical conditions	na	6 naive, 4 free for ≥1 year, 8 free for ≥20 days	PANSS: 78.6 (20.6)	PANSS: 21.7 (7.1)	PANSS: 17.1 (5.9)

[1]SZ=schizophrenia, SZD=schizo-affective disorder

[2]merged patient sample including antipsychotic untreated and treated patients

[3]no significant difference between number of smokers in healthy and patients group

[4]includes all subjects from Laruelle et al. (1996), Abi-Dargahm et al. (1998) and 10 new subjects

[5]The AMPT data for these subjects is reported in Abi-Dargham et al. (2000)

AMPT=alpha-methyl-*para*-tyrosine, PANSS=Positive And Negative Syndrome Scale, FE=first episode of psychosis, BPRS=Brief Psychiatric

Rating Scale, CASH=Comprehensive Assessment of Symptoms and History , Chronic=multiple episode of psychosis, DSM=Diagnostic and Statistical Manual

eTable 3. Methodological characteristics of the studies of dopamine transporter availability

Author	PET Tracer	Radiotracer delivery	Scanner Type	Resolution (FWHM mm)	Outcome Measure	Reference region
Arakawa et al 2009[19]	[^{11}C]PE2I	bolus	ECAT EXACT HR+	Na	BP_{ND}	Cerebellum
Hisao et al 2003[20]	[^{99m}Tc]TRODAT-1	bolus	Siemens Multi-SPECT 3	Na	BP_{ND}	occipital cortex
Laakso et al 2000[21]	[^{18}F]CFT	bolus	ECAT 931/08-12(CTI)	Na	BP_{ND}	Cerebellum
Lavalaye et al 2001[22]	[^{18}F]CFT	bolus	ECAT 931/08-12(CTI)	Na	BP_{ND}	Cerebellum
Laruelle et al 2000[23]	[^{123}I]β-CIT	bolus	Picker PRISM 3000	9-11	BP_{ND}+1	occipital cortex
Lavalaye et al 2001[22]	[^{123}I]FP-CIT	bolus	Na	7.6	BP_{ND}	occipital cortex
Mateos et al 2005[24]	[^{123}I]FP-CIT	bolus	Helix, G.E.M.S.	10	BP_{ND}+1	occipital cortex
Mateos et al 2007[25]	[^{123}I]FP-CIT	bolus	Helix, G.E.M.S.	10	BP_{ND}+1	occipital cortex
Yang et al 2004[26]	[^{99m}Tc]TRODAT-1	bolus	GE Sigma CV-I	Na	BP_{ND}+1	cerebellum
Yoder et al 2004[27]	[^{11}C]β-CFT	bolus	Siemens ECAT 951R, EXACT HR+ (CTI)	Na	BP_{ND}	cerebellum
Schmitt et al 2008[28]	[^{99m}Tc]TRODAT-1	bolus	Picker PRISM 3000	Na	BP_{ND}	cerebellum

eTable 4. Subject characteristics of the studies of dopamine transporter availability

Authors	Controls		Patients									
	N (m/f)	Age mean (sd)/yr	N (m/f)	Age mean (sd)/yr	Diagn-osis [1]	Diagnostic inclusion criteria	Exclusion Criteria	Illness duration	Antipsychotic treatment	Total symptom score (mean [sd])	Positive symptom score (mean [sd])	Negative symptom score (mean [sd])
Arakawa et al 2009	12 (10/2)	33.2 (12.0)	8 (6/2)	36.5 (9.5)	All SZ	DSM-IV	substance abuse, brain disease or epilepsy	32.1 months	6 naïve, 2 free for >6 months	PANSS: 77.8 (18.8)	PANSS: 17.8 (4.8)	PANSS: 18.9 (6.5)
Hisao et al 2003	12 (2/10)	29.8 (8.6)	12 (2/10)	25.9 (7.7)	All SZ	DSM-IV	age <16 or >45 years old, other DSM-IV axis I diagnosis, substance abuse or dependence, severe medical conditions	0.8 years	12 naïve	na	na	Na
Laakso et al 2000	9 (6/3)	29.9 (5.6)	9 (6/3)	30.1 (7.0)	All SZ	DSM-III-R	Na	9 months	9 naïve	na	na	Na
Lavalaye et al 2001	8 (na)	35.3 (5.7)	8 (na)	37.1 (5.7)	All SZ	DSM-IV	Na	119 months	All on AP Tx.	na	na	Na
Laruelle et al 2000	22 (20/2)	39.0 (8.0)	24 (22/2)	41.0 (8.0)	All SZ	DSM-IV	age <18 or >55 years old, other DSM-IV axis I diagnosis, substance abuse or dependence, severe medical conditions	15 years	8 free for mean (sd)=18 (11) days, 16 on AP Tx.	na	na	Na
Lavalaye et al 2001	10 (7/3)	20.3 (0.5)	10 (9/1)	22.1 (3.7)	9 SZ, 1 SZD	DSM-IV	Na	33.5 months	10 naïve	na	PANSS: 22.8 (3.8)	PANSS: 18.9 (6.7)
Mateos et al 2005	10 (6/4)	27.0 (4.3)	20 (14/6)	26.0 (4.8)	All SZ	DSM-IV	CNS medications, CNS disorder, bipolar disorder, substance dependence	4.5 months	All on AP Tx.*	na	PANSS: 27.8(5.3)[#] 27.4(4.5)[$]	PANSS: 25.8 (4.3)[#] 24.4 (7.3)[$]
Mateos et al 2007	15 (8/7)	29.0 (7.0)	20 (14/6)	26.0 (5.0)	All SZ	DSM-IV	CNS medication, CNS disorder, bipolar disorder, substance dependence, positive drug screen (except for cannabis)	4 months	20 naïve*	na	PANSS: 28.25(9.43)[#] 30.75(3.84)[$]	PANSS: 22.63(6.50)[#] 24.17(8.71)[$]
Yang et al 2004	12 (9/3)	33.3 (12.9)	11 (6/5)	26.3 (10.2)	All SZ	DSM-IV	any medical or CNS diseases/head injury, antipsychotic, ECT, or lithium treatment, substance dependence	1.3 years	11 naïve	PANSS: 63.8 (10.8)	na	Na
Yoder et al 2004	10 (7/3)	45.0 (18.3)	10 (8/2)	40.5 (na)	All SZ	DSM-IV	Na	na	1 naïve, 1 free for 1 month, 8 on AP Tx	na	na	Na
Schmitt et al 2008	12 (9/3)	30.5 (7.98)	20 (18/2)	29.3 (6.51)	All SZ	DSM-IV/ICD-10	neuroleptic or antidepressant treatment, alcohol or illegal drug abuse, CNS comorbidity	na	20 naïve	na	PANSS: 30.65 (7.65)	PANSS: 29.50 (6.45)

SZD=schizo-affective disorder, CNS=central nervous system; *Patients were grouped by whether they showed antipsychotic-induced parkinsonism ($^{\#}$) or not ($^{\$}$) at the point of scanning or, in the case of antipsychotic naïve patients, to subsequent antipsychotic treatment

eTable 5. Methodological characteristics of the studies of dopamine receptor availability

	Author	PET Tracer	Radiotracer delivery	Scanner Type	Resolution (FWHM mm)	Outcome Measure	Reference region
Butyrophenones	Crawley et al 1986[29]	[^{76}Br]Bromospiperone	Bolus	IGE 400AT gamma camera	Na	BP_{ND}+1	Cerebellum
	Martinot et al 1990[30]	[^{76}Br]Bromospiperone	Bolus	LETI TTVO1	Na	BP_{ND}+1	Cerebellum
	Tune et al 1993[31]	[^{11}C]NMSP	Bolus	NeuroECAT PET	Na	B_{max}	Cerebellum
	Nordström et al 1995[32]	[^{11}C]NMSP	Bolus	Scanditronix PC 2048-15B	Na	B_{max}	Cerebellum
	Okubo et al 1997[33]	[^{11}C]NMSP	Bolus	PCT3600W40	Na	k_3	Cerebellum
Benzamides	Farde et al 1990[34]	[^{11}C]Raclopride	Bolus	PC-384-7B	Na	B_{max}	Cerebellum
	Hietala et al 1994[35]	[^{11}C]Raclopride	Bolus	ECAT 931/08-12	Na	B_{max}	Cerebellum
	Breier et al 1997[13]	[^{11}C]Raclopride	bolus+ infusion	GE Advance scanner	Na	BP_{ND}	Cerebellum
	Talvik et al 2006[36]	[^{11}C]Raclopride	Bolus	ECAT EXACT 47	4	BP_{ND}	Cerebellum
	Kegeles et al 2010[18]	[^{11}C]Raclopride	bolus+ infusion	ECAT EXACT HR+	4.1	BP_{ND}	Cerebellum
	Pilowsky et al 1994[37]*	[^{123}I]IBZM	Bolus	SME 810 SPECT brain scanner	7-9	BP_{ND}+1	frontal cortex
	Pedro et al 1994[38]*	[^{123}I]IBZM	Bolus	SME 810 SPECT brain scanner	na	BP_{ND}+1	frontal cortex

	Laruelle et al 1996[12]	[^{123}I]IBZM	bolus + infusion	PRISM 3000	11	BP_f	occipital cortex
	Knable et al 1997	[^{123}I]IBZM	Bolus	CERASPECT	11.5	BP_{ND}	occipital cortex
	Abi-Dargham et al 1998[14]	[^{123}I]IBZM	bolus + infusion	PRISM 3000	11	BP_f	occipital cortex
	Yang et al 2004[26]	[^{123}I]IBZM	Bolus	Na	Na	BP_{ND}+1	Cerebellum
	Corripio et al 2006[39]	[^{123}I]IBZM	Bolus	Helix, GEMS	Na	BP_{ND}+1	occipital cortex
	Abi-Dargham et al 2000[17]	[^{123}I]IBZM	bolus + infusion	PRISM 3000 XP	11	BP_{ND}	average of frontal and occipital regions
	Schmitt et al 2009[40]	[^{123}I]IBZM	Bolus	PRISM 3000 XP	Na	BP_{ND}	frontal cortex
	Kessler et al 2009[41]	[^{18}F]Fallypride	Bolus	GE Advance scanner	Na	BP_{ND}	Cerebellum
	Kegeles et al 2010[42]	[^{18}F]Fallypride	Bolus	ECAT EXACT HR+	Na	BP_{ND}	Cerebellum
Ergot derivatives	Martinot et al 1991[43]	[^{76}Br]Bromolisuride	Bolus	LETI TTVO1	Na	BP_{ND}+1	Cerebellum
	Martinot et al 1994[44]	[^{76}Br]Bromolisuride	Bolus	LETI TTVO1	na	BP_{ND}+1	Cerebellum

FWHM=full width half maximum; *there is potential subject overlap between these studies (attempts to contact the authors failed)—the meta-analysis is repeated excluding one study on www.schizoprenia.com.

eTable 6. Subject characteristics of the studies of dopamine receptor availability

Method	Authors	Controls		Patients									
		N (m/f)	Age mean (sd)/yr	N (m/f)	Age mean (sd)/yr	Diag-noses[1]	Diagnostic inclusion criteria	Exclusion Criteria	Illness duration (mean unless stated)	Antipsychotic treatment	Total symptom score (mean [sd])	Positive symptom score (mean [sd])	Negative symptom score (mean [sd])
Butyrophenones	Crawley et al 1986	13 (11/2)	41.2 (10.3)	12 (10/2)	44.3 (18.2)	11 SZ, 1 PD	na	Na	13.4 years	4 naïve, 8 free for ≥ 4 months	na	na	Na
	Martinot et al 1990	12 (na)	28.7 (10.3)	12 (12/0)	28.7 (8.7)	All SZ	DSM-III	age<18 year, female, patient unable to remain medication free for a week prior to scan	na	9 naïve, 3 free for > 1 year	na	CPRS: 42.6 (29.8)	CPRS: 57.6 (25.1)
	Tune et al 1993	17 (13/4)	39 (5.93)	25 (17/8)	34.88 (7.08)	All SZ	DSM-III-R	stroke, mental retardation, significant head trauma, seizure disorder, past ECT, stroke	8.16 years	18 naïve, 7 free for ≥ 4 months	BPRS: 47.2 (5.9)	BPRS: 13.0 (0.94)	BPRS: 7.08 (0.61) SANS: 37.79 (22.66)
	Nordström et al 1995	7 (7/0)	27.7 (6.8)	7 (5/2)	28.4 (5.7)	4 SZ, 3 SZD	DSM-III-R	physically healthy/history of organic brain disorder, head injury, alcohol or drug abuse	≥ 2 months	7 naïve	BPRS: 33 (4)	na	Na
	Okubo et al 1997	18 (na)	27.7 (5.6)	17 (na)	27.4 (5.9)	All SZ	ICD-10	Na	≥ 4 months	10 naïve, 7 free for ≥ 2 weeks	na	na	Na
Benzamides	Farde et al 1990	20 (10/10)	27.5 (4.9)	18 (10/8)	24.2 (3.3)	All SZ	DSM-III	organic brain disorder/ head injury, drug or alcohol abuse,	Median: 10 months[#]	18 naïve	CPRS subscale: 12.0 (3.7)	na	Na
	Hietala et al 1994	10 (6/4)	26.8 (7.3)	13 (9/4)	25.2 (6.8)	All SZ	DSM-III-R	long-term intensive psychotherapy, serious somatic illness	18.7 months	13 naïve	BPRS: 51.4 (18.9)	na	Na
	Breier et al 1997	12 (9/3)	29.2 (SE:2.6)	11 (8/3)	32.4 (SE:3.0)	All SZ	DSM-IV	drug dependence or significant drug abuse, severe head trauma, significant medical condition	6.6 years	6 naïve, 5 free for ≥ 14 days	BPRS: 28.8 (7.2)	na	Na
	Pilowsky et al 1994	20 (11/9)	31.0 (7.8)	20 (11/9)	31.0 (8.5)	All SZ	DSM-III-R	primary substance use disorder, serious physical illness	36 months	17 naïve, 3 free for > 5 years	na	na	Na
	Pedro et al 1994	15 (9/6)	33 (na)	12 (6/6)	33.5 (9.7)	All SZ	DSM-III-R	primary substance use disorder, serious physical illness	4.02 years	10 naïve, 2 free for ≥ 6 months	BPRS: 56.3 (10.2)	BPRS: 22.25 (7.07)	BPRS: 8.5 (5)
	Laruelle et al 1996	15 (14/1)	41 (SE: 2)	15 (14/1)	42 (SE:2)	All SZ	DSM-IV	other DSM-IV axis I diagnosis, substance abuse or dependence, severe medical condition	14 years	1 naïve, 14 free for ≥21 days	BPRS: 37(3)	PANSS: 16.6 (1.7)	PANSS: 14.9 (1.5)

	Knable et al 1997~	16 (11/5)	28.8 (7.8)	21 (18/3)	35.8 (9.0)	19 SZ, 2 SZD	DSM-IV	Na	14.5 years	1 naïve, 20 free for mean=25.6 days~	na	na	na
	Abi-Dargham et al 1998	15 (12/3)	40 (11)	15 (12/3)	41 (9)	All SZ	DSM-IV	other DSM-IV axis I diagnosis, substance abuse or dependence, severe medical conditions	17 years	2 naïve, 13 free for ≥22 days	BPRS: 44 (11)	PANSS: 18.5 (5.1)	PANSS: 19.6 (7.0)
	Abi-Dargham et al 2000	18 (11/7)	31 (8)	18 (11/7)	31 (8)	All SZ	DSM-IV	other DSM-IV axis I diagnosis, substance abuse or dependence, severe medical conditions	na	8 naïve, 10 free for 139 days (mean)	PANSS: 71 (12) (naïve) 63 (11) (free)	na	na
	Yang et al 2004	12 (9/3)	33.26 (12.93)	11 (6/5)	26.25 (10.22)	All SZ	DSM-IV	medical/ neurological diseases, ECT, lithium treatment, alcohol or substance dependence, or head injury	1.3 years	11 naïve	PANSS: 63.8 (10.8)	na	na
	Corripio et al 2006	18 (10/8)	24.2 (4.4)	11 (6/5)	25.6 (4.5)	All SZ	DSM-IV	substance abuse, neurological disease	na	11 naïve	PANSS: 71.1(11.4)	na	na
	Talvik et al 2006	17 (13/4)	Na	18 (9/9)	28.8 (10.5)	All SZ	DSM-IV	psychiatric comorbidity, head injury, drug addiction	≥ 1 year	18 naïve	PANSS: 80.4 (20.9)	PANSS: 21.9 (4.6)	PANSS: 20.1(9.6)
	Schmitt et al 2009	10 (5/5)	32.4 (12.73)	23 (19/4)	28.2 (6.23)	19 SZ, 2 SZD, 2 BP	DSM-IV/ICD-10	na	na	23 naïve	BPRS: 73.6 (na)	PANSS: 29.1 (na)	PANSS: 29.1 (na)
	Kessler et al 2009	11 (5/6)	31.6 (9.2)	11 (6/5)	30.5 (8.0)	All SZ	DSM-IV	significant medical conditions, substance abuse	na	4 naïve, 7 free for ≥ 3 weeks	BPRS (6 item scale): 28.8 (7.0)	SAPS: 9.8 (3.1)	SANS: 9.4 (4.0)
	Kegeles et al 2010	18 (13/5)	29 (7)	18 (13/5)	29 (8)	All SZ	DSM-IV	weight <50kg or> 115kg, other DSM-IV axis I diagnosis, substance abuse or dependence, severe medical conditions	na	6 naïve, 12 free for ≥ 20 days	PANSS: 78.61 (20.63)	PANSS: 21.72 (7.12)	PANSS: 17.17 (5.99)
	Kegeles et al 2010	22 (17/5)	26 (6)	21 (14/7)	31 (12)	All SZ	DSM-IV	medical illness, other DSM-IV Axis I diagnosis, substance abuse	na	5 naïve, 16 free for 191 days (mean)	PANSS: 64 (15)	na	na
Ergot derivatives	Martinot et al 1991	14 (14/0)	23 (4)	19 (12/7)	Men: 22(4) Female: 24(6)	All SZ	DSM-III	age <18 years old, schizophrenic disorder, unable to remain medication free for 1 week before scan	na	10 naive, 9 free for ≥ 6 months	na	na	na
	Martinot et al 1994	10 (na)	21 (2)	10 (na)	20 (2)	All SZ	DSM-III-R:undifferentiated/disorganised sub-types, SANS score>55	Age<18 or >25 years old, marked positive symptoms, lifetime neuroleptic exposure >1 month, unable to remain medication free for 1 week before scan	na	8 naïve, 2 free for ≥ 4 months	na	SAPS: 19.1 (13.8)	SANS: 87.2 (14.2)

PD: Psychotic depression; SZD: Schizo-affective disorder; BP: Brief Psychotic disorder; CPRS: Comprehensive Psychopathological Rating Scale; BPRS: Brief Psychiatric Rating Scale; PANSS: Positive And Negative Syndrome Scale; SAPS: Scale for the Assessment of Positive Symptoms; SANS: Scale for the Assessment of Negative Symptoms; ECT=electro-convulsive therapy

#=mean duration of illness was 1.9 years including the prodrome to the first psychotic episode, range: 1-72 months[45]; ~excluded from the main analysis because the antipsychotic wash-out 7 days in some patients

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Review

Effect of BDNF val^{66}met polymorphism on declarative memory and its neural substrate: A meta-analysis

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ABSTRACT

Brain derived neurotrophic factor (BDNF) is a critical component of the molecular mechanism of memory formation. Variation in the BDNF gene, particularly the rs6265 (*val^{66}met*) single nucleotide polymorphism (SNP), has been linked to variability in human memory performance and to both the structure and physiological response of the hippocampus, which plays a central role in memory processing. However, these effects have not been consistently reported, which may reflect the modest size of the samples studied to date. Employing a meta-analytic approach, we examined the effect of the BDNF *val^{66}met* polymorphism on human memory (5922 subjects) and hippocampal structure (2985 subjects) and physiology (362 subjects). Our results suggest that variations in the rs6265 SNP of the BDNF gene have a significant effect on memory performance, and on both the structure and physiology of the hippocampus, with carriers of the *met* allele being adversely affected. These results underscore the role of BDNF in moderating variability between individuals in human memory performance and in mediating some of the neurocognitive impairments underlying neuropsychiatric disorders.

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1. Introduction

Human declarative memory function has a heritability of about 50% (McClearn et al., 1997). This suggests that naturally occurring genetic variations (Egan et al., 2003; de Quervain and Papassotiropoulos, 2006) may account for a large proportion of the variance in this fundamental cognitive function. Impairments in memory function are a key feature of many neuropsychiatric disorders, including schizophrenia and mood disorders (Aleman et al., 1999; Torres et al., 2007). Among the genes that are known to affect human memory function, the gene coding for the brain derived neurotrophic factor (BDNF) is of particular interest because of the critical role played by the BDNF protein in regulating the structure and function of neurons, including those involved in memory formation (Bekinschtein et al., 2007, 2008). In the hippocampus, a region that is central to declarative memory formation (Milner et al., 1998), BDNF has been shown to be essential and sufficient for the induction of long-term potentiation (Pang et al., 2004; Pastalkova et al., 2006), a form of synaptic plasticity that underlies memory formation (Bliss et al., 1993) as well as for the persistence of memories that have already been formed (Bekinschtein et al., 2007).

BDNF is a member of the neurotrophin family (Reichardt, 2006). It is expressed throughout the brain in cortical and subcortical areas (Yan et al., 1997), particularly in the hippocampus (Murer et al., 2001). Selective alterations in hippocampal BDNF expression have been reported during and after hippocampus-dependent learning (Mizuno et al., 2000). The gene coding for BDNF (Liu et al., 2005) is located at chromosome 11. It codes for a precursor peptide (pre-pro-BDNF), which is successively cleaved to generate pro-BDNF and mature BDNF, both of which are secreted and extracellularly active (Pang et al., 2004). The only functional single nucleotide polymorphism (SNP) identified in the BDNF gene (OMIM 113505) results in a *methionine* (*met*) to *valine* (*val*) substitution at codon 66 in the pro-region of BDNF (rs6265). The *met* variant of the precursor peptide has been associated with impaired intracellular trafficking of pro-BDNF into dendrites and vesicles as well as a reduction in activity-dependent secretion, the process that plays a major role in the regulation of extracellular levels of BDNF (Egan et al., 2003). These functional effects of the SNP appear to have several deleterious consequences for hippocampal structure and function.

Consistent with evidence from genetic manipulation studies (Mizuno et al., 2000; Heldt et al., 2007), impairments in episodic memory performance (Egan et al., 2003; Hariri et al., 2003; Dempster et al., 2005a), and alterations in hippocampal structure (Pezawas et al., 2004; Szeszko et al., 2005; Bueller et al., 2006; Nemoto et al., 2006; Schofield et al., 2009) and function (Hariri et al., 2003; Hashimoto et al., 2008) have been reported in human carriers of the *met* allele. However, it is unclear to what extent functional variation in the BDNF gene associated with the *val*^{66}*met* polymorphism underlies inter-individual variability in human declarative memory function, and in the structure and physiology of the hippocampus. Not all studies have consistently reported an effect of BDNF *val*^{66}*met* polymorphism on memory performance (Strauss et al., 2004; Zivadinov et al., 2007; Matsuo et al., 2009) and hippocampal structure (Dutt et al., 2009; Joffe et al., 2009). Moreover, whether the effects of BDNF on grey matter volume are restricted to the hippocampus or are part of a more general effect on grey matter volume across the brain is unclear (Toro et al., 2009). The inconsistency across studies may reflect limited sample sizes, as well as variations between studies in the clinical diagnosis (Ho et al., 2006; Chepenik et al., 2009; Lau et al., 2010; Matsuo et al., 2009; Cerasa et al., 2010), gender (Ozan et al., 2010) and age (Nemoto et al., 2006; Li et al., 2010) of the subjects. A better understanding of the effects of variations in the BDNF gene on memory function is also important because of the role of BDNF and memory impairments in the major psychiatric disorders, including schizophrenia (Muglia et al., 2003), bipolar disorder, depression (Strauss et al., 2009), and anxiety (Chen et al., 2006).

Meta-analytic methods provide a way to statistically integrate the results from a large number of separate studies, thereby improving the power to detect significant effects, as well as the influence of potential confounding factors. In this study, we conducted three separate meta-analyses to examine the extent to which inter-individual variability in human memory function is a function of variation in the BDNF gene and whether that variability may reflect the effects of the BDNF gene on the structure and function of the hippocampus, the key neural substrate for declarative memory. We also report the influence of potential confounding factors on the effect of the BDNF polymorphism such as clinical diagnosis, laterality, age, gender or publication bias.

2. Methods

2.1. Search strategy and selection of studies

The PubMed database was searched and all studies reporting effects of the *val*^{66}*met* polymorphism on performance in declarative memory tasks, hippocampal volume and hippocampal activation until the 1st of August 2011 were included, regardless of gender, ethnicity or diagnostic group. For the literature search we used a combination of search terms describing the BDNF gene ("BDNF" or "brain-derived" or "neurotrophic factor") and search terms describing the BDNF-polymorphism ("val^{66}met" or "rs6265" or "polymorphism") and restricted the time of publication to the 1st of August 2011. For the meta-analysis of episodic memory, we added search terms describing hippocampal memory function ("memory" or "hippocampus"). For the meta-analysis of hippocampal volume and hippocampal physiology we used the same initial search terms by adding the search term describing the hippocampus ("hippocampal" or "hippocampus" or "MRI").

We excluded studies that investigated patients with neurological disorders. In order to investigate the effect of the *val*^{66}*met* polymorphism in context of a psychiatric diagnosis but also in healthy subjects, we included psychiatric as well as healthy populations. All studies that examined the effect of the BDNF gene on hippocampal physiology employing functional MRI (fMRI) were included, irrespective of the specific cognitive processes that were examined, as only a limited number of studies have reported this in the context of a declarative memory task. The bibliographies of the selected publications were hand-searched for further studies. As the *met* homozygote variant is rare in the general population, most studies merged *met* homozygotes with *val*/*met* heterozygotes to compare *val* homozygotes with *met* carriers.

2.2. Data extraction

For each selected study, the following information was extracted: publication (names of the authors, publication year), sample characteristics (sample size, Hardy–Weinberg-equilibrium, diagnostic group, gender, age, medication, *val* and *met* carriers). In general, measures (mean of memory performance, mean of hippocampal volume, mean of hippocampal response, size of genotyping groups, *p*-values, *t* statistic, *F* statistic) that allowed us to quantify the effect-size (cohen's *d*) were extracted.

The main outcome measure for the first meta-analysis was the standardized mean difference (SMD) between *val* and *met* carriers in performance during memory tasks. In order to maximize the statistical power of the analysis and to ensure optimal use of all the available data, we initially included all studies reporting the effects

of BDNF *val*^{66}*met* polymorphism on declarative memory tasks. As a range of different memory tasks were used in these studies, we carried out additional analyses including only studies that employed an identical memory task [Wechsler Memory Scale-revised (WMS-R)] resulting in a more homogeneous group. This allowed us to examine whether the effect evident in the more inclusive first-level meta-analysis was consistently replicated in the subsequent analyses that included more homogeneous studies.

The main outcome measures for the second and third meta-analyses respectively were SMD in hippocampal grey matter volume and task-related change in hippocampal blood oxygen-level dependent (BOLD) response ('hippocampal-response') as measured by fMRI. If information published with the studies was not sufficient to calculate a mean effect-size between *val*/*val* and *met* carriers, authors were contacted and asked to supply further data. If sufficient data could not be obtained, studies were not included in the meta-analysis. In case the Hardy–Weinberg equilibrium (HWE) was not reported, it was computed from the given allele frequencies using the χ^2-test.

2.3. Data analysis

Statistical analysis of the extracted data was conducted using the R statistical programming language version 2.10.1 with the package 'metafor' (Viechtbauer, 2010; R Development Core Team, 2012). The individual effect-size for each study was entered into a random-effects model as this approach does not assume between-study homogeneity in effect-size and therefore allows inferences to be drawn from a potentially heterogeneous group of studies that are valid for the whole population (Hedges et al., 1985; Hedges and Vevea, 1998). The heterogeneity across studies was assessed by the inconsistency parameter I^2 (Higgins et al., 2003). The summary effect-sizes (cohen's d) were computed using a restricted maximum-likelihood estimator. In order to test for publication bias resulting from a greater likelihood of positive results getting published, visual inspection of funnel plots was carried out for each meta-analysis followed by linear regression analysis to test for funnel plot asymmetry (Egger's test) (Egger et al., 1997). In case of potential bias by selective publication as indicated by the Egger's test, a trim-and-fill approach was used (Duval and Tweedie, 2000). This method identifies potentially missing studies in a funnel-plot and corrects summary effect-size estimates by adding missing studies. Meta-regression analyses were run including publication year, diagnosis, gender and age as factors to evaluate the source of heterogeneity in the effect-sizes and to check the influence of potential confounding variables. In case studies reported F-statistics from ANOVA with three groups (val/val-, val/met- and met/met-carrier), we corrected the F-value in order to be comparable with F-values obtained employing a 2-way ANOVA following the formula: $F_{\text{two-way}} = F_{\text{three-way}} \cdot ((n-2)/(n-3))$, with n representing the total sample size of all three groups combined.

3. Results

3.1. Effect of the val^{66}met polymorphism on declarative memory performance

We detected 134 potential studies that were screened according to our inclusion criteria. One hundred and six studies were excluded as either no episodic memory performance was reported, no BDNF-genotypes were reported, patients with a neurological disorder were investigated, no original data was reported (review article or meta-analysis), no human population was investigated (animal study) or there was an overlap in the investigated sample with other studies already included in the meta-analysis (see Fig. 1). Therefore

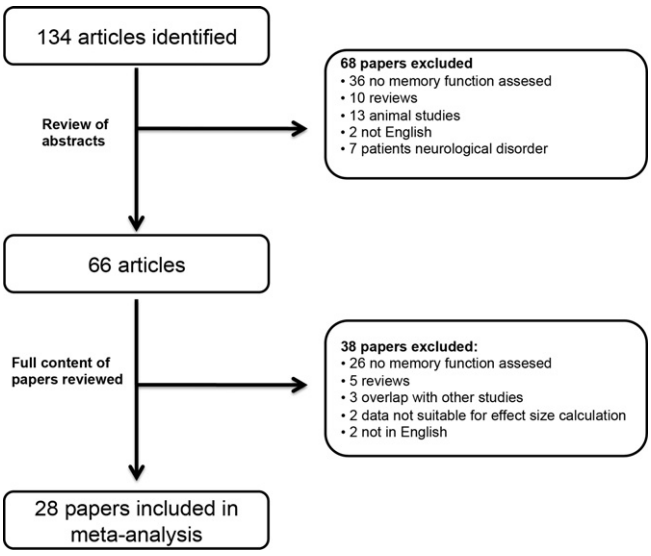


Fig. 1. Flow-chart of describing the number of studies excluded at each step for the meta-analysis of association studies of the *val*^{66}*met* polymorphism and declarative memory performance.

28 studies published between 2003 and 2011 matched the search criteria ((Hariri et al., 2003; Egan et al., 2003; Strauss et al., 2004; Tan et al., 2005; Dempster et al., 2005b; Harris et al., 2006; Ho et al., 2006; Hashimoto et al., 2008; Miyajima et al., 2008; Raz et al., 2008; Li et al., 2009; Matsuo et al., 2009; Schofield et al., 2009; Gong et al., 2009; Baig et al., 2010; Benjamin et al., 2010; Karnik et al., 2010; Cathomas et al., 2010; Richter-Schmidinger et al., 2011; Sambataro et al., 2010; van Wingen et al., 2010; Cerasa et al., 2010; Chung et al., 2010; Dennis et al., 2011; Kanellopoulos et al., 2011; Laing et al., 2011; Voineskos et al., 2011; Gruber et al., 2012), see Table 1). From the remaining studies, data were extracted from 32 independent samples resulting in a final sample of 5922 subjects. No study showed significant deviation from the HWE equilibrium.

The random-effects analysis revealed a summary effect-size of $d = 0.16$ (95%-CI: 0.08–0.23, $z = 4.0785$, $p < 0.0001$, $I^2 = 34.98\%$, 95%-CI for I^2: 6.07–77.43%), with the *met* carriers performing worse than the *val* homozygotes (see Fig. 2a). However there was evidence for a potential publication bias ($z = 2.2464$, $p = 0.0247$, Fig. 2b). In order to account for this potential bias trim-and-fill was carried out revealing $n = 6$ potentially missing studies. In the corrected model there was a significant summary effect size of $d = 0.1$ (95%-CI: 0.01–0.2, $z = 2.0872$, $p = 0.04$, $I^2 = 61.87\%$, 95%-CI for I^2: 47.18–77.43%). Meta-regression with year of publication revealed no significant effect (beta = −0.0095, $F(1,30) = 0.2833$, $p = 0.5985$, see Fig. 2c). Further regression analysis showed no effect of the potential confounding factors such as age, sex ratio or met carrier ratio (all $p > 0.1$).

In order to address the heterogeneity between the investigated studies arising from the inclusion of patient and healthy samples as well individuals with different psychiatric diagnoses, we carried out further sensitivity analyses using more homogeneous subgroups of studies. Random-effects analysis with 21 samples of only healthy subjects ($n = 4262$) showed an effect size of $d = 0.15$ (95%-CI: 0.06–0.23, $z = 3.2069$, $p = 0.001$, $I^2 = 35.35\%$, 95%-CI for I^2: 0–77.43%). However, the Egger's test ($z = 2.2748$, $p = 0.0229$) suggested potential publication bias. In order to account for this potential bias trim-and-fill was carried out revealing $n = 6$ potentially missing studies. In the corrected model there was a non-significant trend for the summary effect-size: $d = 0.09$ (95%-CI: 0–0.18, $z = 1.865$, $p = 0.06$, $I^2 = 42.6\%$, 95%-CI for I^2: 15.24–77.43%). Random-effects analysis with only the patient sample (11 samples: $n = 1660$) showed a significant effect size of $d = 0.2$ (95%-CI: 0.01–0.39, $z = 2.055$, $p = 0.04$,

Table 1
Studies included in the meta-analysis of the effect of the *val*^{66}*met* polymorphism and declarative memory performance.

Study		Population					Allele frequency		Memory test
First author	Year	Diagnosis	*n*	Age	Male	Female	val/val	met-carriers	
Egan	2003	Healthy	641	36.21	363	278	435	206	WMS-R logical memory test (delayed recall)
Hariri	2003	Healthy	28	30.6	16	12	14	14	Recall complex scenes
Strauss	2004	Depression/ dysthymia	62	18.4	na	na	43	19	WMS-R logical memory test (delayed recall)
Dempster	2005	Healthy	114	na	na	na	84	30	WMS-R logical memory test (delayed recall)
Tan	2005	Schizophrenia	108	21.7	70	38	19	89	WMS-R logical memory total score
Harris	2006	Elderly	460	79	186	274	303	157	WMS-R logical memory test (delayed recall)
Ho	2006	Healthy	144	27.9	65	79	95	49	Verbal memory battery composite score
Ho	2006	Schizophrenia	293	27.39	213	80	182	111	Verbal memory battery composite score
Hashimoto	2008	Healthy	58		12	46	17	41	WMS-R logical memory test (delayed recall)
Miyajima	2008	Elderly	722	63	na	na	471	251	Recall word list
Raz	2008	Healthy	103	53.24	30	73	62	41	WMS-R logical memory test (delayed recall)
Gong	2009	Healthy	679	na	na	na	202	477	Recall word list
Li	2009	Healthy	110	na	na	na	64	46	WMS-R logical memory test (delayed recall)
Matuso	2009	Mixed[a]	84	35.37	24	60	53	31	California Verbal Learning Test 2
Schofield	2009	Healthy	475	32.4	233	242	282	193	Verbal list learning
Baig	2010	Healthy	58	26.23	23	35	39	19	Verbal memory task
Benjamin	2010	Mixed[b]	264	69.6	110	154	171	93	WMS-R logical memory test (delayed recall)
Cathomas	2010	Healthy	333	22.8	101	232	203	130	recall word list
Cerasa	2010	Healthy	32	30.51	15	17	14	18	Rey Auditory Verbal Learning Test (delayed recall)
Chung	2010	Schizophrenia (violent)	47	38.5	47	0	14	33	Rey Auditory Verbal Learning Test (delayed recall)
Chung	2010	Schizophrenia (non-violent)	48	37.9	48	0	14	34	Rey Auditory Verbal Learning Test (delayed recall)
Dennis	2010	Healthy	22	23.55	11	11	11	11	Relational memory task
Kanellopoulos	2010	Depressed	33	72.31	12	21	17	16	Hopkins Verbal Learning Test (delayed recall)
Karnik	2010	Healthy	149	na	na	na	99	50	WMS-R logical memory test (immediate recall)
Richter-Schmidinger	2010	Healthy	135	24.56	44	91	84	51	Inventar zur Gedächtnisdiagnostik (delayed recall)
Sambataro	2010	Healthy	125	40.95	60	65	80	45	Recall complex scenes
van Wingen	2010	Healthy	47	na	na	na	32	15	Face recognition
Gruber	2011	Healthy	39	na	na	na	23	16	VLMT[c]
Gruber	2011	Schizophrenia	40	na	na	na	21	19	VLMT[c]
Gruber	2011	Bipolar	40	na	na	na	21	19	VLMT[c]
Laing	2011	Healthy	360	72.7	189	171	248	112	Verbal list learning
Voineskos	2011	Healthy	69	46	44	25	41	28	RBANS[d]

[a] Subjects with borderline disorder and healthy controls.
[b] Subjects with major depressive disorder and healthy controls.
[c] Verbal Learning Memory Test.
[d] Repeatable battery for the assessment of the neuropsychological status.

I^2 = 58.15%, 95%-CI for I^2: 0–77.43%) and no evidence for a publication bias (z = 0.2998, p = 0.7643).

Random-effects analysis including all the studies that employed the same memory task (WMS-R) (9 studies including patients and healthy subjects; n = 1920), resulted in a non-significant trend for a summary effect-size of d = 0.22 (95%-CI: −0.04 to 0.48, z = 1.6582, p = 0.1, I^2 = 82.81%, 95%-CI for I^2: 57.63–77.43%) with no evidence for a publication bias (z = 1.3889, p = 0.1648).

3.2. Effect of the *val*^{66}*met* polymorphism on hippocampal volume

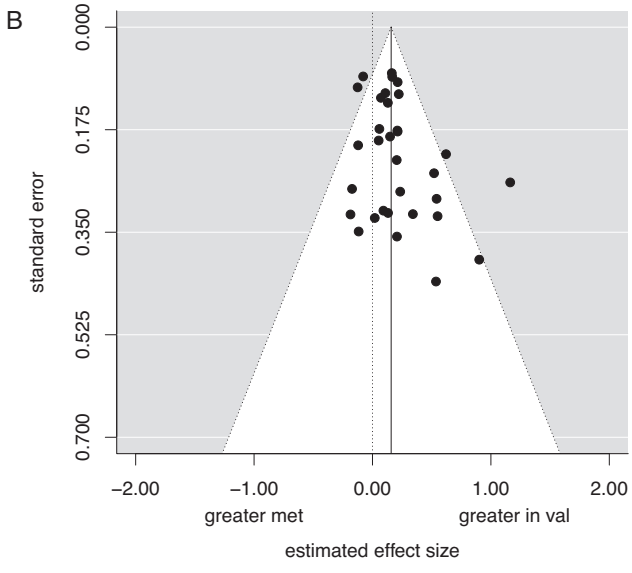
We detected 108 potential studies that were screened according to our inclusion criteria. Eighty-four studies were excluded as either no hippocampal volume was reported, no BDNF-genotypes were reported, no original data was reported (review article or meta-analysis), only post-mortem data was reported or no human population was investigated (animal study). Among those, three further studies were excluded as the data reported did not allow computation of effect size estimated and contacting the authors for additional information was unsuccessful (see Fig. 3). Therefore 24 studies published between 2004 and 2011 matched the search criteria ((Pezawas et al., 2004; Szeszko et al., 2005; Bueller et al., 2006; Nemoto et al., 2006; Frodl et al., 2007; Miyajima et al., 2008; Montag et al., 2008; Stern et al., 2008; Takahashi et al., 2008; Joffe et al., 2009; Koolschijn et al., 2010; Thomason et al., 2009; Toro et al., 2009; Chepenik et al., 2009; Dutt et al., 2009; Jessen et al., 2009; Benjamin et al., 2010; Karnik et al., 2010; Richter-Schmidinger et al., 2011; Soliman et al., 2010; Cerasa et al., 2010; Gonul et al., 2011; Cole et al., 2011; Gruber et al., 2012), see Table 2). From these, we extracted data from 35 independent samples, resulting in a final sample of 2985 subjects. No study showed significant deviation from the HWE equilibrium. Analysis employing the random-effects model led to a summary effect-size d = 0.12 (95%-CI: −0.01 to 0.26, z = 1.8621, p = 0.06, I^2 = 62.56%, 95%-CI for I^2: 44.04–82.63%, see Fig. 4a) that was significant at a trend level, with a smaller hippocampal volume in *met* carriers as compared to the *val* homozygotes.

However, the Egger's test (z = 3.1628, p = 0.0016, see Fig. 4b) suggested that there was potential publication bias. In order to account for this potential bias, trim-and-fill analysis was carried out revealing n = 9 potentially missing studies. The corrected model showed a non-significant effect-size of d = −0.03 (95%-CI: −0.19 to 0.12, z = −0.4393, p = 0.7, I^2 = 76.81%, 95%-CI for I^2: 68.36–82.63%).

Investigation of the potential sources for this bias in the conducted meta-analysis revealed a significant decrease in effect-size over the years (beta = −0.11, $F(1,33)$ = 8.8554, p = 0.0054, see Fig. 4c). In order to estimate the summary effect-size accounting for this effect of year of publication, a mixed-model was calculated with year of publication as a covariate. The model accounting for the covariate showed a summary effect-size of d = 0.11 (95%-CI: 0–0.21, z = 1.9586, p = 0.05, I^2 = NA%, 95%-CI for I^2: NA to 81.16%). Further meta-regression analysis revealed no effects of the factors age, gender ratio, *met* carrier ratio and diagnosis (all p > 0.1).

A

study	year	d	95%–CI upper	95%–CI lower	z–score	p–value
Egan	2003	0.1668	0.0008	0.3328	1.9693	0.0489
Hariri	2003	0.9016	0.1241	1.6791	2.2727	0.0230
Strauss	2004	−0.1724	−0.7132	0.3684	−0.6249	0.5320
Dempster	2005	0.6213	0.1967	1.0459	2.8681	0.0041
Tan	2005	1.1631	0.6440	1.6821	4.3919	<0.0001
Harris	2006	−0.1255	−0.3264	0.0755	−1.2237	0.2211
Ho	2006	0.2100	−0.1356	0.5556	1.1910	0.2336
Ho	2006	0.0713	−0.1648	0.3074	0.5920	0.5539
Hashimoto	2008	0.5414	−0.0325	1.1153	1.8490	0.0645
Miyajima	2008	0.1633	0.0099	0.3167	2.0865	0.0369
Raz	2008	−0.1211	−0.5160	0.2738	−0.6011	0.5478
Gong	2009	−0.0788	−0.2434	0.0858	−0.9385	0.3480
Li	2009	0.0523	−0.3266	0.4312	0.2704	0.7869
Matuso	2009	0.2048	−0.2395	0.6490	0.9033	0.3663
Schofield	2009	0.2126	0.0290	0.3962	2.2698	0.0232
Baig	2010	0.2341	−0.3159	0.7841	0.8344	0.4041
Benjamin	2010	0.1299	−0.1229	0.3826	1.0070	0.3139
Cathomas	2010	0.1086	−0.1117	0.3289	0.9660	0.3340
Cerasa	2010	0.2067	−0.4935	0.9070	0.5786	0.5628
Chung	2010	0.5498	−0.0823	1.1818	1.7048	0.0882
Chung	2010	−0.1857	−0.8119	0.4406	−0.5811	0.5612
Dennis	2010	0.5364	−0.3142	1.3870	1.2359	0.2165
Kanellopoulos	2010	−0.1167	−0.8000	0.5665	−0.3349	0.7377
Karnik	2010	0.0581	−0.2820	0.3982	0.3347	0.7378
Richter–Schmidinger	2010	0.2121	−0.1367	0.5610	1.1918	0.2333
Sambataro	2010	0.1485	−0.2172	0.5142	0.7960	0.4261
van Wingen	2010	0.0923	−0.5213	0.7059	0.2948	0.7681
Gruber	2011	0.0195	−0.6186	0.6575	0.0598	0.9524
Gruber	2011	0.1314	−0.4899	0.7526	0.4145	0.6785
Gruber	2011	0.3413	−0.2838	0.9663	1.0700	0.2846
Laing	2011	0.2221	−0.0016	0.4458	1.9459	0.0517
Voineskos	2011	0.5201	0.0318	1.0083	2.0876	0.0368
RE model		**0.1577**	**0.0819**	**0.2334**	**4.0785**	**<0.0001**



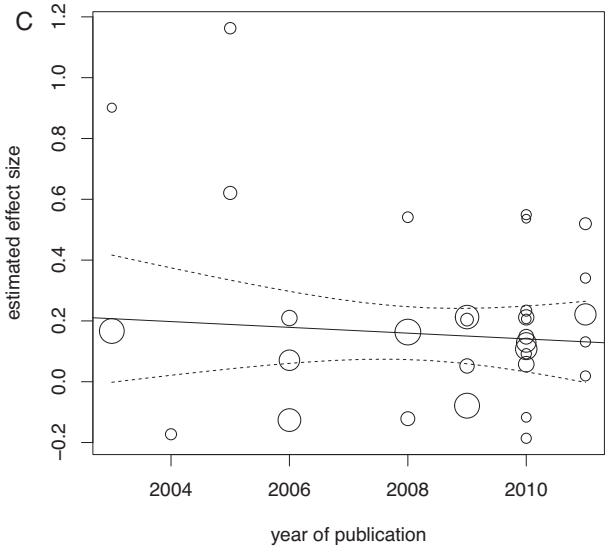


Fig. 2. (a) Meta-analysis (random-effects model) of association studies of the *val*^{66}*met* polymorphism and declarative memory performance. Position of the boxes represents the effect-size of each study, with the size of the box proportional to the size of the study. 95% CI are indicated by error bars. At the bottom of the figure a summary effect-size across all the studies is shown, (b) funnel plot of studies investigating effects of the *val*^{66}*met* polymorphism on declarative memory, (c) meta-regression of the effect of the *val*^{66}*met* polymorphism on declarative memory and year of publication.

Table 2
Studies included in the meta-analysis of the effect of the *val*^{66}*met* polymorphism and hippocampal volume.

Study		Population			Gender		Allele frequency		Normalization			
First author	Year	Diagnosis	*n*	Age	Male	Female	val/val	met-carriers	Brain volume	Age	Gender	Scanner type
Pezawas	2004	Healthy	111	32.5	56	55	69	42	+	+	+	1.5 T
Szeszko	2005	Mixed[a]	44	26.2	24	20	27	17	+	–	+	1.5 T
Bueller	2006	Healthy	36	27	14	22	21	15	+	–	–	1.5 T
Nemoto	2006	Healthy	109	36.2	38	71	41	68	–	–	–	1.5 T
Frodl	2007	Healthy	60	41.6	29	31	37	23	+	–	–	1.5 T
Frodl	2007	Depression	60	44.2	29	31	40	20	+	–	–	1.5 T
Miyajima	2008	Healthy	61	na	na	na	43	18	+	–	–	1.5 T
Montag	2008	Healthy	87	23.91	24	63	54	33	+	+	+	1.5 T
Stern	2008	Healthy	50	30.52	33	17	38	12	+	–	–	3.0 T
Takahashi	2008	Healthy	29	24.2	17	12	13	16	+	–	–	1.5 T
Takahashi	2008	Schizophrenia	33	25.6	20	13	12	21	+	–	–	1.5 T
Chepenik	2009	Healthy	18	21–56	6	12	12	6	+	+	+	1.5 T
Chepenik	2009	Bipolar	20	18–58	9	11	12	8	+	+	+	1.5 T
Dutt	2009	Schizophrenia	128	36.16	82	46	89	39	–	–	–	1.5 T
Dutt	2009	At risk[b]	193	48.19	81	113	136	58	–	–	–	1.5 T
Dutt	2009	Healthy	60	40.79	28	33	44	17	–	–	–	1.5 T
Jessen	2009	Healthy	30	na	na	na	24	6	+	–	–	1.5 T
Jessen	2009	Depression	79	48.2	27	52	47	32	+	–	–	1.5 T
Joffe	2009	Healthy	113	na	na	na	68	45	–	+	+	1.5 T
Koolschijn	2009	Healthy	90	38.19	56	34	59	31	+	–	–	1.5 T
Koolschijn	2009	Schizophrenia	87	36.05	71	16	55	32	+	–	–	1.5 T
Thomason	2009	Healthy	29	na	9	20	17	12	+	–	–	3.0 T
Toro	2009	Healthy	331	12–19	159	172	217	114	–	–	–	1.0 T
Benjamin	2010	Mixed[c]	264	69.6	110	154	171	93	+	+	+	1.5 T
Cerasa	2010	Healthy	155	40.25	59	96	99	56	+	+	+	1.5 T
Gonul	2010	Healthy	40	29.76	17	23	24	16	+	+	+	1.5 T
Gonul	2010	Depression	33	33.75	8	25	15	18	–	–	–	1.5 T
Karnik	2010	Healthy	129	49.3	59	70	87	42	+	–	–	1.5 T
Richter-Schmidinger	2010	Healthy	135	24.52	44	91	91	84	–	–	–	1.5 T
Soliman	2010	Healthy	70	24.9	36	34	35	35	+	–	–	3.0 T
Cole	2011	Healthy	111	33	55	56	68	41	+	–	–	1.5 T
Cole	2011	Depression	84	48.82	27	57	47	32	+	–	–	1.5 T
Gruber	2011	Healthy	39	na	na	na	24	15	+	–	–	1.5 T
Gruber	2011	Schizophrenia	33	na	na	na	20	13	+	–	–	1.5 T
Gruber	2011	Bipolar	34	na	na	na	16	18	+	–	–	1.5 T

[a] Healthy controls and patients with schizophrenia.
[b] Relatives of subjects with schizophrenia.
[c] Healthy controls and patients with depression.

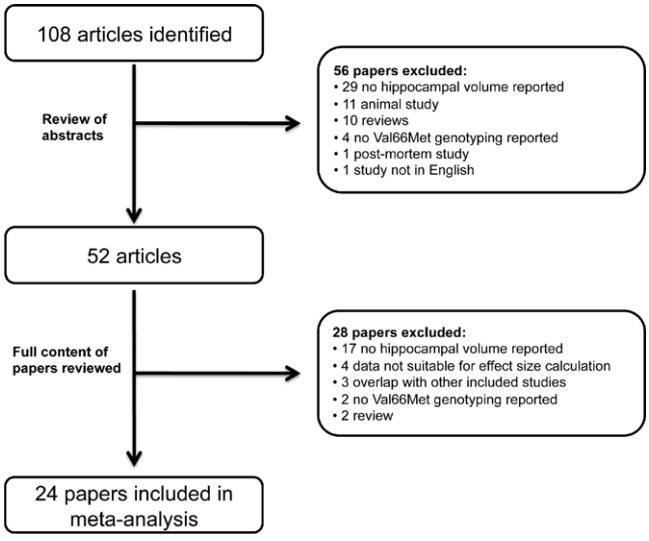


Fig. 3. Flow-chart of describing the number of studies excluded at each step for the meta-analysis of association studies of the *val*^{66}*met* polymorphism and hippocampal grey matter volume.

In order to further address the issue of heterogeneity between studies, we carried out a sensitivity analysis, first with the sub-group of studies that reported the effects in healthy subjects and then a further sub-group analysis of those studies that normalized the hippocampal volume measures to total brain volume. There was no significant effect of diagnosis (healthy vs patient population) or normalization to total intracranial volume on effect-size estimates (see supplementary results). Analysis in a more homogenous sample of only healthy subjects whose hippocampal volumes had been normalized with regard to total intracranial volume (14 studies, 939 subjects) revealed a significant effect size of $d = 0.25$ (95%-CI: 0.02–0.47, $z = 2.1139$, $p = 0.03$, $I^2 = 61.09\%$, 95%-CI for I^2: 22.74–82.63%) and no evidence for a publication bias ($z = 1.4302$, $p = 0.1527$) or year of publication (beta = −0.099, $F(1,11) = 3.5404$, $p = 0.0866$). Further analyses of samples restricting the analyses to studies ($n = 31$) that employed a MR scanner of similar field strength (1.5 T), only healthy subjects and only patients resulted in effect-size estimates in the similar range as that obtained with the total sample (see supplementary results).

3.3. *Effect of the val*^{66}*met polymorphism on hippocampal activation*

We detected 107 potential studies that were screened according to our inclusion criteria. Ninety-seven studies were excluded as either no hippocampal activation was reported, no BDNF-genotypes were reported, no original data was reported (review article or meta-analysis) or no human population was investigated (animal study). Therefore, 10 studies published between 2003 and 2011 matched the search criteria. They examined hippocampal physiology as measured by the blood oxygen level dependent (BOLD) response, employing paradigms that engaged a wide-range of cognitive processes, including episodic memory, working

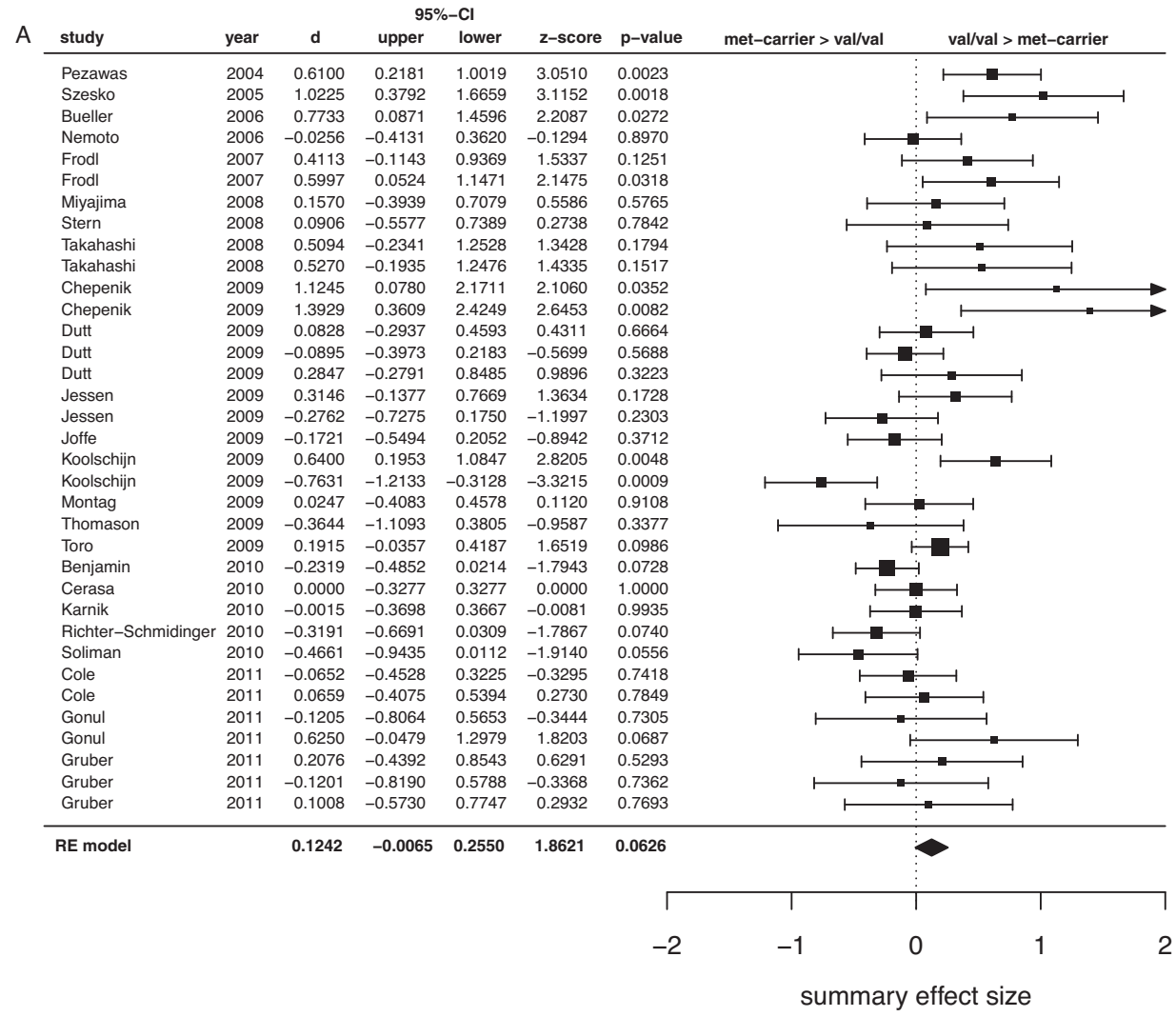


Fig. 4. (a) Meta-analysis (random-effects model) of association studies of the *val*^{66}*met* polymorphism and hippocampal grey matter volume. Position of the boxes represents the effect-size of each study, with the size of the box proportional to the size of the study. 95% CI are indicated by error bars. At the bottom of the figure a summary effect-size across all the studies is shown, (b) funnel plot of studies investigating effects of the *val*^{66}*met* polymorphism on hippocampal volume, (c) meta-regression of the effect of the *val*^{66}*met* polymorphism on hippocampal volume and year of publication.

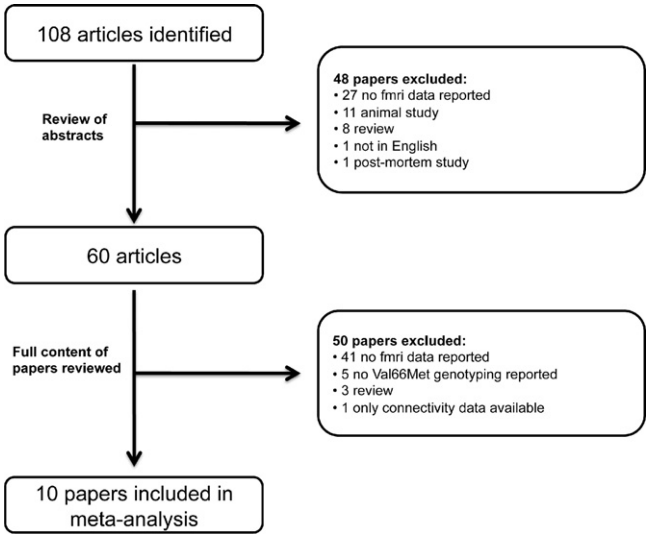


Fig. 5. Flow-chart of describing the number of studies excluded at each step for the meta-analysis of association studies of the *val*^{66}*met* polymorphism and hippocampal activation.

memory, decision-making, and emotional processing. From those, we extracted data from 12 independent samples resulting in a final sample of 362 subjects ((Egan et al., 2003; Hariri et al., 2003; Hashimoto et al., 2008; Gasic et al., 2009; Lau et al., 2010; Schofield et al., 2009; Cerasa et al., 2010; Dennis et al., 2011; van Wingen et al., 2010; Banner et al., 2011), see Table 3). No study showed significant deviation from the HWE.

A random-effects model showed a summary effect-size of $d = 0.59$ (95%-CI: 0.01–1.16, $z = 2.0106$, $p = 0.0444$, $I^2 = 82.84\%$, 95%-CI for I^2: 65.12–94.3%, see Fig. 6a). Funnel plot analysis of the studies revealed no evidence for a publication bias ($z = 0.7820$, $p = 0.4342$, see Fig. 6b). Meta-regression with year of publication revealed no significant effect (beta = −0.1114, $F(1,10) = 1.0982$, $p = 0.3193$). There was no effect of the factors gender-ratio or ratio of *met* carriers ($p > 0.1$). There was a non-significant trend for an effect of mean age of the studied population (beta = 0.065, $F(1,10) = 4.0762$, $p = 0.0711$) with older populations exhibiting stronger effects of the polymorphism on hippocampal response (Figs. 4–6).

Restricting the sample to only healthy populations (11 samples, 335 subjects) showed a significant summary effect-size of $d = 0.69$ (95%-CI: 0.09–1.28, $z = 2.2479$, $p = 0.02$, $I^2 = 82.59\%$, 95%-CI for I^2: 63.49–94.3%) with no evidence for a publication bias ($z = 0.7133$, $p = 0.4756$).

In order to further address the issue of heterogeneity between studies, arising as a result of different cognitive paradigms employed by the studies included in this meta-analysis, we carried out a sensitivity analysis that was restricted to the subset of studies that employed a memory task (either episodic or working memory; 7 samples; 216 subjects). The random-effects model show a non-significant summary effect-size of $d = 0.53$ (95%-CI: −0.48 to 1.53, $z = 1.0316$, $p = 0.3023$, $I^2 = 88.09\%$, 95%-CI for I^2: 65.15–94.3%) with no evidence for a publication bias ($z = -0.8033$, $p = 0.4218$).

4. Discussion

We conducted three separate quantitative reviews using a meta-analytic approach to investigate the effect of the *val*^{66}*met* polymorphism of the BDNF gene on declarative memory function in humans, and on the structure and physiology of the hippocampus. We found evidence that declarative memory performance, hippocampal volume and hippocampal activation are all reduced in carriers of the *met* allele compared to val homozygotes. These results point to a modest but consistent role of this polymorphism in mediating the individual variability in hippocampal structure, activation and hippocampus-mediated cognitive functioning. As it is unlikely that BDNF is the only gene that mediates the individual variability in hippocampal structure (Callicott et al., 2005; Tan et al., 2011), activation and memory functioning (de Frias et al., 2004; Kauppi et al., 2011), the modest effect-sizes noted here, nevertheless may reflect a biologically meaningful role of this BDNF polymorphism in mediating inter-individual variability in this specific cognitive function and its underlying neural substrate. This is consistent with the evidence from basic research indicating the role of BDNF in memory function (Bekinschtein et al., 2007, 2008) and the hippocampus (Pang et al., 2004; Pastalkova et al., 2006).

Random-effects analysis of the effect of the *val*^{66}*met* polymorphism on hippocampus-mediated declarative memory function revealed a small effect-size ($d = 0.16$) that was not explained by potential confounding variables such as age, gender, diagnosis or *met* carrier status. Moreover, restricting the analysis to studies employing the same declarative memory task revealed an even larger effect of variation in BDNF gene on memory performance, despite the sample being smaller (but more homogeneous). However we cannot exclude the possibility that the results of the main meta-analysis of memory function might have been biased by the different tests used to assess memory. Those differ with respect to the type of information being stored (verbal information or complex scenes) or the duration of storage (long-term or short-term) and thus might measure related but not identical cognitive constructs. The effect-size in the total sample of $d = 0.16$ corresponds to 0.64% of the variance in memory performance, slightly smaller than the effect (2%) of the gene coding for the catechol-o-methyltransferase (COMT) enzyme on memory (de Frias et al., 2004) and smaller than the 5% that has been attributed to variation in a cluster of seven memory-associated SNPs (adenylyl cyclase, PKA, CAMKII, NMDA receptor, metabotropic glutamate receptor

Table 3
Studies included in the meta-analysis of the effect of the *val*^{66}*met* polymorphism and hippocampal activation.

Study		Population					Allele frequency		Method	
First author	Year	Diagnosis	*n*	Age	Male	Female	val/val	met-carriers	Scanner type	Task
Egan	2003	Healthy	13	38.80	10	3	8	5	1.5/3.0 T	n-back
Egan	2003	Healthy	17	29.90	10	7	12	5	1.5/3.0 T	n-back
Hariri	2003	Healthy	28	30.60	16	12	14	14	3.0 T	Encoding/retrieval of complex scenes
Hashimoto	2008	Healthy	58	36.40	12	46	17	41	1.5 T	Encoding/retrieval of complex scenes
Schofield	2008	Healthy	37	30.14	19	18	20	17	1.5 T	Oddball-task
Gasic	2009	Healthy	29	28.86	16	13	21	8	3.0 T	Relative preference task
Banner	2010	Healthy	21	na	na	na	16	5	1.5 T	Spatial navigation memory task
Cerasa	2010	Healthy	32	30.45	15	17	18	14	1.5 T	n-back
Dennis	2010	Healthy	22	22.55	11	11	11	11	1.5 T	Relational memory task
Lau	2010	Healthy	31	13.71	14	17	23	8	3.0 T	Emotional pictures
Lau	2010	Depression	27	13.44	12	15	18	9	3.0 T	Emotional pictures
van Wingen	2010	Healthy	47	38.00	13	34	32	15	1.5 T	Encoding/retrieval of face pictures

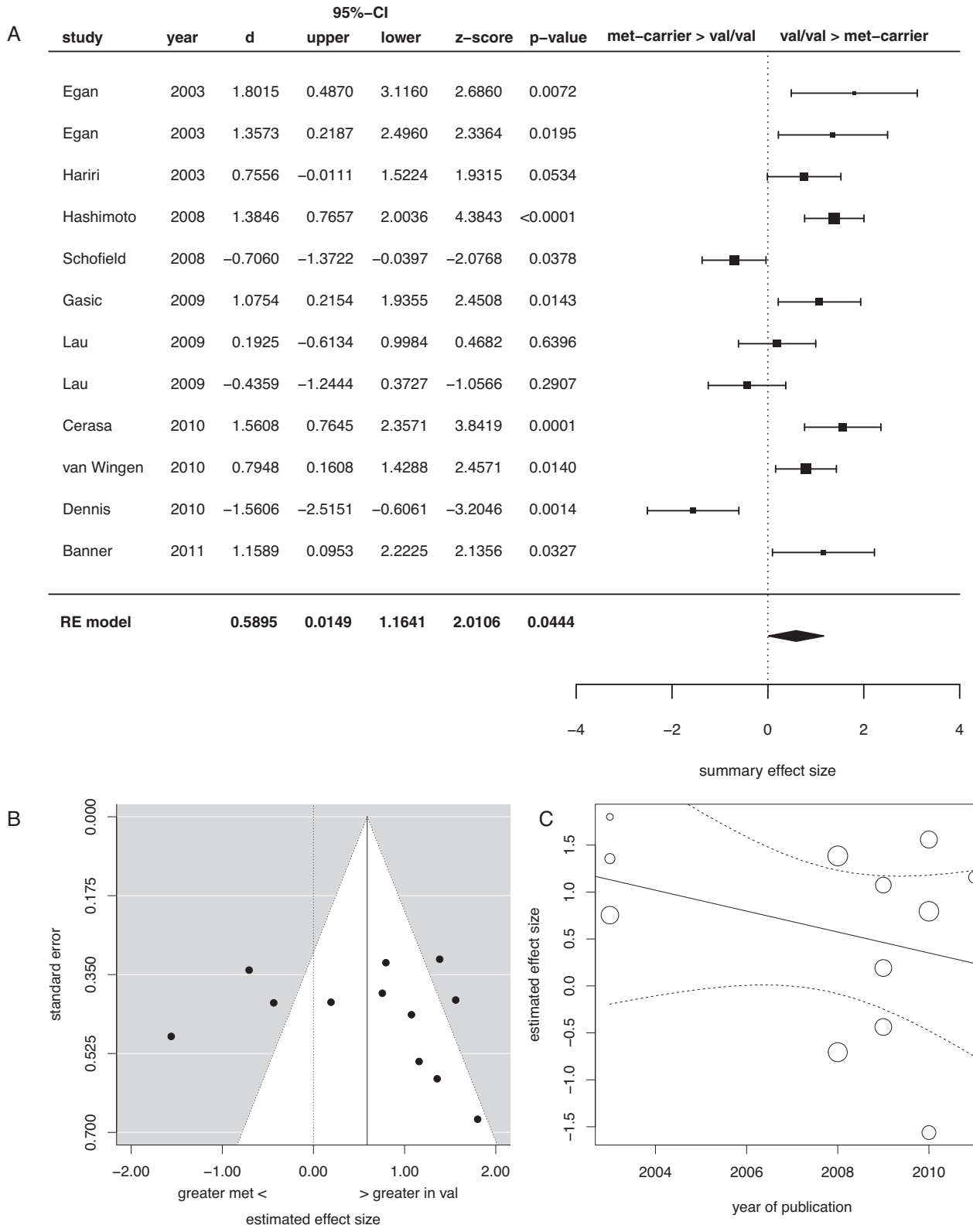


Fig. 6. (a) Meta-analysis (random-effects model) of association studies of the *val*^{66}*met* polymorphism and hippocampal activation. Position of the boxes represents the effect-size of each study, with the size of the box proportional to the size of the study. 95% CI are indicated by error bars. At the bottom of the figure a summary effect-size across all the studies is shown, (b) funnel plot of studies investigating effects of the *val*^{66}*met* polymorphism on hippocampal activation.

and PKC) (de Quervain and Papassotiropoulos, 2006). It is unclear whether the effect of BDNF on memory function is the result of a general effect on cognitive performance rather than a domain-specific effect on declarative memory processing, as it may also affect working memory performance (Echeverria et al., 2005; Egan et al., 2003; Rybakowski et al., 2003, 2006; Zivadinov et al., 2007) and cognitive processing speed (Miyajima et al., 2008). However, studies investigating the effect of the *val*^{66}*met* polymorphism on

general cognitive ability as measured by the IQ have not shown a consistent effect (Egan et al., 2003; Hansell et al., 2007). As the majority of studies investigated patients with schizophrenia who received antipsychotic medication, this might represent a further potential confound in our analysis as antipsychotic medication has been shown to affect memory function (Riedel et al., 2010).

We then examined whether the effect of genetic variation in BDNF on declarative memory performance could be accounted for by its effect on the structure of the hippocampus, the principal neural substrate for declarative memory processing. A recent meta-analysis reported significant effects of the BDNF polymorphism on hippocampal volume (Hajek et al., 2012). However this analysis was based on a comparably small sample as authors restricted their analysis to only healthy subjects and manual tracings of hippocampal volumina.

Following a more comprehensive approach we only found a small non-significant effect ($d = 0.12$) on hippocampal volume. Restricting the analysis to studies ($n = 17$) that reported hippocampal volume normalized to ICV did not change the magnitude of the effect ($d = 0.14$).

However restricting our analysis to a more homogenous sample of only healthy controls samples with the hippocampal volume normalized to total intracranial volume revealed a significant effect size ($d = 0.25$) with no evidence for a publication bias or effect of year of publication. This effect is slightly smaller than the effect size of $d = 0.41$ found in a comparable analysis on a more restrictive sample (Hajek et al., 2012).

The magnitude of the effect of variation in the BDNF gene on hippocampal volume that we have reported needs to be considered in light of heritability estimates of between 40 and 69% for hippocampal volume (Peper et al., 2007) and a relatively large effect ($d = 0.39$) of the COMT gene on hippocampal grey matter volume (Ehrlich et al., 2010). It has also been argued that the effect of the *val*^{66}*met* BDNF polymorphism on hippocampal grey matter is a reflection of its effect on total brain volume (Toro et al., 2009), consistent with the ubiquitous expression of the gene in the brain. BDNF risk allele carriers have been shown to exhibit grey matter volume changes in regions beyond the hippocampus like the parahippocampal gyrus (Nemoto et al., 2006; Sublette et al., 2008; Takahashi et al., 2008; Gatt et al., 2009; Montag et al., 2009), the amygdala (Sublette et al., 2008; Takahashi et al., 2008; Gatt et al., 2009; Montag et al., 2009) and the frontal lobe (Pezawas et al., 2004; Szeszko et al., 2005; Ho et al., 2006; Takahashi et al., 2008; Varnäs et al., 2008; Toro et al., 2009). A stronger effect-size including only studies (Pezawas et al., 2004; Szeszko et al., 2005; Agartz et al., 2006; Bueller et al., 2006; Nemoto et al., 2006; Frodl et al., 2007; Takahashi et al., 2008; Miyajima et al., 2008; Chepenik et al., 2009; Jessen et al., 2009; Koolschijn et al., 2010; Benjamin et al., 2010; Soliman et al., 2010) that normalized hippocampal volume measures to total ICV suggests a region-specific effect of BDNF *val*^{66}*met* polymorphism on hippocampal volume that is unlikely to be the result of a more generalized effect on total brain volume. It is worth noting that a majority of the patients with schizophrenia that were investigated in the studies reviewed here had received antipsychotic medication. Numerous studies have reported an effect of such treatment on brain morphology in patients with schizophrenia (Smieskova et al., 2009; Ho et al., 2011). Thus antipsychotic treatment might have affected a potential difference in the effect of the BDNF-polymorphism between patients with schizophrenia and healthy controls. Nevertheless, the effects of BDNF *val*^{66}*met* polymorphism on hippocampal volume remain significant when only studies involving healthy controls were included in the meta-analyses.

Finally, we examined whether variations in the BDNF gene have an effect on hippocampal physiology. To address this, we investigated the effect of the *val*^{66}*met* polymorphism on task-related hippocampal response, as measured using fMRI. The random-effects model showed a significant and high effect-size of $d = 0.59$ comparable to another meta-analysis (Munafò et al., 2008) that reported the effect of the 5-HTTLPR polymorphism on amygdala activation ($d = 0.59$). These effects were not attributable to the confounding effects of age, gender, diagnosis or the ratio of *met* carriers. Moreover, when the analysis was restricted to only studies that involved memory tasks, as opposed to other cognitive processes, we found a comparable effect-size ($d = 0.53$) with no evidence for a publication bias. This effect was however smaller compared to that reported for COMT on hippocampal signal change ($d = 1.3$) during a memory task (Bertolino et al., 2006).

In summary, our meta-analyses of the literature indicate that a functional polymorphism of the BDNF gene associated with the *val*^{66}*met* polymorphism significantly modulates declarative memory function, and the structure and physiology of the hippocampus. Effects of age, gender, diagnosis or met-carrier status did not account for these effects. It is possible that we did not observe a significant effect of diagnosis on any of the measures because fewer studies examined patient samples, with only modest sample-sizes. Further, the heterogeneity of psychiatric diagnoses in the patient samples precluded any meaningful estimation of effect-size in patients. However, the separate analyses with only healthy and patient samples attest to the consistency of the results. Overall, these results suggest that a naturally occurring functional variation in the BDNF gene accounts for a significant proportion of the normal inter-individual variation in human memory function. Our meta-analyses were mainly carried out on separate sets of studies of memory performance, hippocampal structure and hippocampal physiology, as opposed to studies that examined all three measures in the same sample. Thus, we were not able to explore the extent to which the effects of BDNF at the behavioural level were accounted for by its effects on hippocampal structure and/or physiology. However, we found that the effect of variation in the BDNF gene on memory performance was weaker than the effect on the physiological response of the hippocampus. This is consistent with the suggestion that measures such as neural physiology may be more proximate and hence more sensitive to the effect of functional genetic polymorphisms on gene products and function, than measures that are more distant such as behaviour (Hariri et al., 2006). However in a comprehensive analysis of a COMT-polymorphism it has been shown that in fact associations with schizophrenia as a psychiatric diagnosis are not weaker than associations with potential endophenotypes (Flint and Munafò, 2007). We examined the effect of BDNF on hippocampal physiology as determined by fMRI while performing a variety of cognitive and emotional processing tasks. Thus, greater effect of BDNF on hippocampal activation as compared to memory performance may simply reflect a greater penetrance of the effect of this polymorphism on general hippocampal physiology, that is not exclusively attributable to its role in memory processing.

It is important to exercise caution in interpreting the significance of these results. Firstly, we identified significant heterogeneity in all three sets of meta-analyses. This may reflect the fact that a complex interaction between multiple potential confounding factors such as other cognitive processes (working memory, IQ, processing speed), other genes, gene–gene and gene–environment interactions may have affected the results presented here. Apart from the BDNF-polymorphism that we investigated, variations in a number of other genes have been shown to affect memory processes and might thus have influenced the results (Papassotiropoulos et al., 2006). It has been suggested that other genetic polymorphisms might interact with secretion or transcription of BDNF and might thus be relevant to the effect of the BDNF-polymorphism (Pezawas et al., 2008). Similarly, potential interactions between genes and environmental factors such as early

life stress may also have affected these results (Gatt et al., 2009). It is also possible that the effect of the BDNF-polymorphism on other cognitive processes that are related to memory function might be a confounding factor in this analysis.

Another important factor that needs to be kept in mind is the role of selective publication as a potential confounder. There is evidence that the reporting of results is biased by positive selection of significant results (Easterbrook et al., 1991). As meta-analyses are restricted to the available data in the form of publications, this might result in an overestimation of the summary effect sizes. In our analysis we have tried to address this problem by carefully investigating the data for evidence of publication bias. In a set of unbiased studies, effect-sizes are assumed to scatter symmetrically around the summary effect-size. Publication bias is assumed to lead to a violation of the symmetry of this distribution of effect-sizes. Therefore, typical meta-analytic procedures include visualization of available studies in funnel-plots and subsequent inspection of symmetry by a regression test (Egger et al., 1997). If there was evidence of publication bias, we used trim-and-fill procedures to estimate the summary effect-size after controlling for publication bias (Peters et al., 2007).

Changes in hippocampus-mediated memory function and in hippocampal activation and volume are considered to be critical in schizophrenia (Tamminga et al., 2010). Abnormal hippocampal activation has also been reported in mood disorders (Lau et al., 2010). Evidence from genetic association studies also point toward a role of variation in the BDNF gene in schizophrenia (Muglia et al., 2003; Nanko et al., 2003), bipolar disorder and depression (Strauss et al., 2009) as well as anxiety (Chen et al., 2006). Moreover psychiatric disorders including schizophrenia have been shown to be associated with decreased BDNF serum levels (Green et al., 2011), reduced BDNF expression in the hippocampal formation (Iritani et al., 2003) and variations in the BDNF gene have also been reported to play a role in treatment response to schizophrenia. Results from the meta-analyses presented here suggest how variation in the BDNF gene may mediate critical neurocognitive impairments observed in various neuropsychiatric conditions.

Conflict of interest

There is no competing financial interests in relation to the work described for any of the authors.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.neubiorev.2012.07.002.

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Supplementary RESULTS:

Effect of diagnosis on estimated effect size

Meta-analysis employing the random-effects model that only included studies in healthy subjects (23 samples; 2086 subjects) showed a significant effect size of d=0.13 (95%-CI: 0 to 0.27, z=1.9225, p=0.0499, I2=50.74%, 95%-CI for I2: 17.94 to 82.63%) and evidence for a publication bias (z = 2.1192, p = 0.0341).
A trim-and-fill approach detected n=2 potentially missing studies and after correction the random-effects model showed a non-significant effect size of d=0.06 (95%-CI: -0.09 to 0.2, z=0.7483, p=0.5, I2=60.4%, 95%-CI for I2: 39.61 to 82.63%).

In studies including only patient populations (10 samples; 591 subjects) the summary effect size of the d=0.07 (95%-CI: -0.2 to 0.34, z=0.4939, p=0.6214, I^2=63.22%, 95%-CI for I^2: 25.72 to 81.16%) and evidence for a publication bias (z = 2.1525, p = 0.0314). Trim-and-fill approch did reveal n=1 any potentially missing studies. The trim-and-fill corrected model showed a non-significant effect size of d=-0.01 (95%-CI: -0.35 to 0.34, z=-0.0322, p=1, I2=73.43%, 95%-CI for I2: 46.39 to 82.63%).

Direct comparisons of the summary effect size between studies using of healthy controls and studies of patients by two-sample t-test was not significant (p>0.1).

Effect of normalization on estimated effect size

As some of the studies reported hippocampal volume measures normalized to the total intracranial volume (ICV) whereas others did not, the meta-analysis was re-run for normalized (21 samples; 1521 subjects) and non-normalized (12 samples; 1316 subjects) studies separately. Employing the random-effects model, this resulted in an effect-size of d=0.14 (95%-CI: -0.06 to 0.35, z=1.4027, p=0.2, I2=69.9%, 95%-CI for I2: 47.96 to 82.63%) and evidence for publication bias (z = 2.6193, p = 0.0088) for the normalized studies. A trim-and-fill approach detected n=3 potentially missing studies and after correction the random-effects model showed a non-significant effect size of d=0.06 (95%-CI: -0.16 to 0.27, z=0.5246, p=0.6, I2=73.69%, 95%-CI for I2: 58.42 to 82.63%).

For the non-normalized studies there was an effect size of d=0.11 (95%-CI: -0.07 to 0.29, z=1.2138, p=0.2, I2=53.74%, 95%-CI for I2: 6.04 to 82.63%) with no evidence for publication bias (z = 1.6706, p = 0.0948).

Direct comparisons of the summary effect size between studies using normalized hippocampal volume and studies using uncorrected measures by two-sample t-test was not significant (p>0.1).

A random-effects model on a subset of studies (14 studies, 939 subjects) that only included healthy subjects and also normalized hippocampal volumes with respect to total brain volume (or total intracranial volume) showed a significant effect size of d=0.25 (95%-CI: 0.02 to 0.47, z=2.1139, p=0.03, I2=61.09%, 95%-CI for I2: 22.74 to 82.63%) and no evidence for a publication bias (z = 1.4302, p = 0.1527) and no effect of year of publication (beta=-0.099, F(1,11)=3.5404, p=0.0866).

Effect of magnetic field strength on estimated effect size

A random-effects model of studies that used scanners with a magnetic field strength of 1.5 T (31 samples, 2505 subjects) showed a significant effect size of d=0.16 (95%-CI: 0.01 to 0.3, z=2.1366, p=0.03, I2=63.31%, 95%-CI for I2: 43.26 to 82.63%) with evidence for a publication bias ($z = 4.6621$, $p < .0001$). A trim-and-fill approach detected n=12 potentially missing studies and after correction the random-effects model showed a non-significant effect size of d=-0.05 (95%-CI: -0.22 to 0.13, z=-0.5224, p=0.6, I2=78.83%, 95%-CI for I2: 70.5 to 82.63%).

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