

# Synapse Pathology in Schizophrenia: A Meta-analysis of Postsynaptic Elements in Postmortem Brain Studies

Amber Berdenis van Berlekom<sup>\*1,2</sup>, Cita H. Muffihah<sup>2,3</sup>, Gijsje J. L. J. Snijders<sup>1,2</sup>, Harold D. MacGillavry<sup>4</sup>, Jinte Middeldorp<sup>2</sup>, Elly M. Hol<sup>2</sup>, René S. Kahn<sup>1,5,6</sup>, and Lot D. de Witte<sup>1,5,6</sup>

<sup>1</sup>Department of Psychiatry, University Medical Center Utrecht Brain Center, Utrecht University, Utrecht, The Netherlands; <sup>2</sup>Department of Translational Neuroscience, University Medical Center Utrecht Brain Center, Utrecht University, Utrecht, The Netherlands; <sup>3</sup>Faculty of Pharmacy, Universitas Muhammadiyah Surakarta, Sukoharjo, Indonesia; <sup>4</sup>Cell Biology, Department of Biology, Faculty of Science, Utrecht University, Utrecht, The Netherlands; <sup>5</sup>Department of Psychiatry, Icahn School of Medicine at Mount Sinai, New York City, NY; <sup>6</sup>Mental Illness Research, Education and Clinical Center (MIRECC), James J Peters VA Medical Center, Bronx, NY

\*To whom correspondence should be addressed; tel: +31-88-75-68811, fax: +31(0)887569032, e-mail: [a.berdenisvanberlekom-2@umcutrecht.nl](mailto:a.berdenisvanberlekom-2@umcutrecht.nl)

Changed synapse density has been suggested to be involved in the altered brain connectivity underlying schizophrenia (SCZ) pathology. However, postmortem studies addressing this topic are heterogeneous and it is not known whether changes are restricted to specific brain regions. Using meta-analysis, we systematically and quantitatively reviewed literature on the density of postsynaptic elements in postmortem brain tissue of patients with SCZ compared to healthy controls. We included 3 outcome measurements for postsynaptic elements: dendritic spine density (DSD), postsynaptic density (PSD) number, and PSD protein expression levels. Random-effects meta-analysis (31 studies) revealed an overall decrease in density of postsynaptic elements in SCZ (Hedges's  $g$ :  $-0.33$ ; 95% CI:  $-0.60$  to  $-0.05$ ;  $P = .020$ ). Subgroup analyses showed reduction of postsynaptic elements in cortical but not subcortical tissues (Hedges's  $g$ :  $-0.44$ ; 95% CI:  $-0.76$  to  $-0.12$ ;  $P = .008$ , Hedges's  $g$ :  $-0.11$ ; 95% CI:  $-0.54$  to  $0.35$ ;  $P = .671$ ) and specifically a decrease for the outcome measure DSD (Hedges's  $g$ :  $-0.81$ ; 95% CI:  $-1.37$  to  $-0.26$ ;  $P = .004$ ). Further exploratory analyses showed a significant decrease of postsynaptic elements in the prefrontal cortex and cortical layer 3. In all analyses, substantial heterogeneity was present. Meta-regression analyses showed no influence of age, sex, postmortem interval, or brain bank on the effect size. This meta-analysis shows a region-specific decrease in the density of postsynaptic elements in SCZ. This phenotype provides an important cellular hallmark for future pre-clinical and neuropathological research in order to increase our understanding of brain dysconnectivity in SCZ.

**Key words:** quantitative review/psychiatry/synapse/CNS

## Introduction

Schizophrenia (SCZ) is a severe psychiatric disorder affecting approximately 0.5%–1% of the general population, causing high morbidity and mortality rates.<sup>1–4</sup> Core symptoms of SCZ are characterized by hallucinations, lack of motivation, and cognitive impairments and are thought to result from altered brain connectivity and network organization.<sup>5–9</sup> Accumulating evidence from genetic and neuropathological studies implies that changes in synapse density underlie these alterations in macroscale connectome organization in SCZ.<sup>10–19</sup> This is supported by studies reporting gray matter volume reductions in SCZ patient brains caused by a decrease in neuropil rather than a loss of cell number.<sup>20–23</sup> Furthermore, it was recently shown that levels of the presynaptic protein synaptophysin are decreased in SCZ hippocampus, frontal cortex, and cingulate cortex.<sup>19</sup> However, a combined systematic analysis of changes in the expression of postsynaptic proteins and the density of postsynaptic elements such as dendritic spines is lacking.

Dendritic spines are small bulges on dendrites, forming the primary site of input for most excitatory synapses in the brain.<sup>24–26</sup> The number of dendritic spines is dynamic, particularly during development, showing a rapid increase during childhood followed by a prominent decrease during adolescence.<sup>27</sup> Interestingly, changes in spine pruning rate during adolescence have been implicated in the development of SCZ.<sup>28–31</sup> Dendritic spines contain many different proteins involved in neurochemical signaling. In particular, neurotransmitter receptor proteins such as NMDARs and AMPARs are anchored in

the postsynaptic density (PSD) by numerous scaffolding proteins such as PSD95<sup>32</sup>. Thus, the PSD has an important role in arranging and coordinating receptor function and is essential for efficient synaptic transmission.

The density of postsynaptic structures in postmortem brain tissue can be determined using several approaches (figure 1A–D). Dendritic spine density (DSD) can be quantified with Golgi staining and immunohistochemistry (IHC) (figure 1B).<sup>33</sup> At the ultrastructural level, electron microscopy studies can identify the number of PSDs that are separated by a synaptic cleft from a presynaptic membrane, forming functional synapses (figure 1C).<sup>34</sup> Also, PSD protein expression levels, measured with western blot or IHC, although varying with the size of the PSD, are thought to reflect the number of synapses (figure 1D).<sup>35,36</sup> Therefore, all these measures (DSD, PSD number, and PSD protein expression), which we collectively refer to as “postsynaptic elements,” can be used as proxies for the number of excitatory synapses in postmortem brain tissue.

Although literature on the density of postsynaptic elements in SCZ is quite extensive, findings are often conflicting. Most postmortem brain studies included a limited number of subjects due to restricted availability of material and the labor intensiveness of performing histological studies. Furthermore, a large variety of premortem and postmortem confounders contributes to the high heterogeneity observed between postmortem studies.<sup>37</sup> In addition, it is difficult to draw conclusions on regional effects as studies are performed using different methodological approaches and assess different brain regions. Altogether, these factors limit the understanding of the contribution of changes in postsynaptic element in the pathophysiology of SCZ.

While literature on DSD, PSD number, and PSD protein expression in SCZ postmortem brain tissue has been reviewed individually,<sup>13–18,38</sup> an integrated assessment combining these different types of synapse density measurements in multiple brain areas in SCZ using meta-analysis has not been performed before. Although not often performed in the context of preclinical studies, meta-analysis provides a powerful tool to synthesize data on a specific topic.

The primary aim of this study was to review the evidence for alterations in the density of postsynaptic elements in SCZ postmortem brain tissue. The second aim was to analyze whether changes in the density of postsynaptic elements are specific to certain brain regions. To this end, we performed a systematic search to qualitatively and quantitatively review available literature on DSD, PSD density, and PSD protein expression in SCZ.

## Methods

### Search Strategy

This quantitative review is performed according to the Preferred Reporting Items for Systematic Reviews and

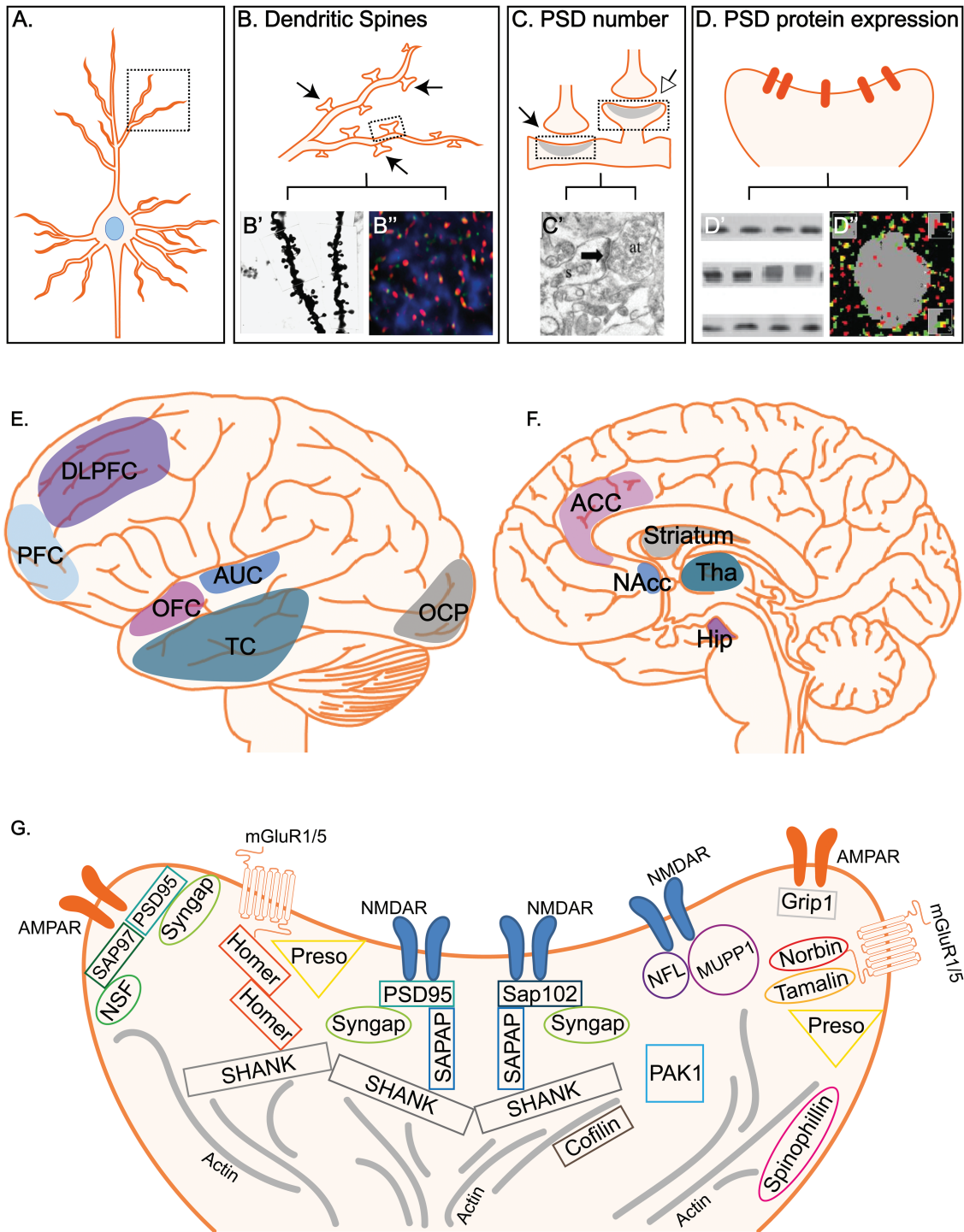
Meta-Analyses (PRISMA),<sup>39</sup> following Meta-analysis of Observational Studies in Epidemiology (MOOSE)<sup>40</sup> guidelines throughout. Two systematic searches were performed in PubMed: (1) (spine OR dendritic spine OR spine\*) AND (density) AND ((Schizophrenia OR Psychosis OR Psychotic)); (2) (schizophreni\* OR psychosis OR schizophrenia) AND (post synapse OR PSD OR post-synapse OR post-synapt\* OR post synapt\* OR postsynap\*). The search was updated until April 30, 2018. Prespecified inclusion criteria were set as: human postmortem studies; comparing patients with healthy controls; measuring a structural outcome of postsynaptic elements (DSD, PSD number, or PSD protein expression); original research, published in a peer-reviewed journal; written in English. Exclusion criteria were: presence of other neurological disorders; animal studies; review articles; reanalysis of previously published data; proteomic/transcriptomic approaches; studies that reported data incompletely and did not provide the information upon request. Furthermore, as messenger ribonucleic acid measurements provide no direct structural readout of the number of postsynaptic elements and posttranslational modifications can result in a poor relation between transcript and protein expression, we excluded studies focusing on RNA only.

### Data Extraction

A.B.v.B. and L.D.W. independently performed title and abstract screening for both systematic searches and reviewed full text for eligibility. Data extraction was performed by A.B.v.B. and checked independently by C.H.M. In addition to main outcome variables (DSD, PSD number, and PSD protein expression), following variables were extracted for effect size (ES) calculation and potential moderator analyses: sample size, methods, brain bank, brain area, subregion, age, sex, post-mortem interval (PMI), and pH. When data records in the original article were not sufficient to generate ES, corresponding authors were asked to provide the raw data. Reference lists were checked for cross-references. In case of follow-up data or reanalysis of previously reported data,<sup>41–44</sup> we only included outcomes of the original research. Studies using partly overlapping samples, studying different brain areas or different proteins, were included separately. Where data were not reported numerically, data were extracted using <https://automeris.io/WebPlotDigitizer/>.

### Quality Control

Methodology, study design, and reporting were assessed to evaluate quality of included studies. Methodology was checked for complete description of technical methods and analyses. Study design was rated by researches blinded to diagnosis, whether they checked for neuropathology, the degree of matching of control and patient population,



**Fig. 1.** Schematic representation of postsynaptic element measurements and brain regions included in the meta-analysis. Panel (A)–(D) show measurements that are used to quantify postsynaptic elements in postmortem brain tissue. (A) shows a neuron with its dendritic tree. The enlargement in (B) shows that each dendrite contains numerous dendritic spines (arrows), which can be quantified using Golgi staining (B' from Glantz and Lewis, 2000<sup>70</sup>) or immunohistochemistry (B'' from Shelton et al, 2015<sup>16</sup>). In (C), presynaptic terminals innervate postsynaptic densities (PSD) on a dendritic spine (white arrow), forming an axospinous synapse, or directly on the dendrite (black arrow), forming an axodendritic synapse. The number of these PSD can be measured with electron microscopy (C' from Roberts et al, 2015). The PSD in (D) is an accumulation of many postsynaptic proteins at the postsynaptic membrane, which can be quantified by western blot (D' from Clinton et al, 2006) or immunohistochemistry (D'' from Chung et al, 2016). (E)–(G) provide a simplified representation of brain regions and proteins in the PSD that are assessed in studies included in our meta-analysis: PFC, prefrontal cortex; DLPFC, dorsolateral prefrontal cortex; OFC, orbitofrontal cortex; OC, olfactory cortex; AC, auditory cortex; TC, temporal cortex; OCP, occipital cortex; ACC, anterior cingulate cortex; Nacc, nucleus accumbens; Tha, thalamus; Hip, hippocampus.

and the assessment/correction of general (age/PMI) and other confounding factors (such as: medication use, suicide, or smoking). For reporting, we assessed whether studies fully described the method of psychopathological examination, population demographics, and main outcome variables.

### Statistical Analysis

Meta-analyses were performed using the Comprehensive Meta-Analysis software (Biostat). Change in DSD, PSD number, or PSD protein expression per brain (sub)region was used to quantify ES between SCZ and the control group. Sample size, mean, and standard deviation (SD) were used to generate ES. When mean and/or SD were unavailable, sample size and exact *P* value were used to generate ES. Hedges's *g* and the upper/lower limit of the 95% CI were used to express ES. A random-effects model was used as heterogeneity between studies was to be expected. Heterogeneity between studies was measured with Cochran's *Q*-test and *I*<sup>2</sup> statistic to provide an estimation of the variation attributed to differences in true effects. *Q* (weighted sum of squares) is equal to *df* if studies share a common effect. *I*<sup>2</sup> reflects the proportion of observed variance reflecting real differences in ES by dividing the excess dispersion (*Q* - *df*) by the total dispersion (*Q*). *I*<sup>2</sup> was considered low at 25%, moderate at 50%, and high at 75%. Publication bias was assessed by visual inspection of the funnel plot and calculated with Egger's test (significance level: *P* < .1). Random-effects meta-regression analyses were performed to analyze the role of potential confounding factors (brain bank, age, sex, and PMI). As we expected that different measurements within the same study are not independent of each other, we nested data from these studies in a conservative approach, computing combined scores from all measurement within one study.

The primary meta-analysis was performed pooling all included studies to assess a brain-wide effect on the density of postsynaptic elements in SCZ. We further stratified the analysis with subgroup analysis of a priori selected variables, analyzing biological (subcortical/cortical) and technical variation (outcome measures), to assess sources of heterogeneity. Data of the same study were included in multiple subcategories when data were reported separately for these categories (indicated with \*). As we assume a common among-study variance across different subgroups, we pooled within group estimates of tau-squared. Between-group differences were tested using the *Q*-test based analysis of variance to determine whether the variance within subgroups was significantly smaller than the variance of all the combined data ( $Q_{\text{between}} = Q_{\text{total}} - (Q_{\text{SubgroupA}} + Q_{\text{SubgroupB}})$ ). Exploratory subgroup meta-analysis, separating data based on subbrain area, were performed when at least 5 independent studies (recommended for random-effects meta-analysis) could be included.<sup>45</sup> Throughout the study, forest plot figures show random-effects meta-analysis,

representing ES in Hedges's *g* with 95% CI for each study. Square size is proportional to study weight and the gray diamond indicates pooled effect size. Schematic images were produced using Motifolio.

## Results

### Database Search

Database searches in PubMed and cross-referencing yielded a total of 1527 records (figure 2). After title and abstract screening, 116 studies remained for full text assessment. Of these, we excluded 81 studies (supplementary table 1). Authors of 4 studies<sup>46-49</sup> were contacted for additional information; a reply was received from one.<sup>49</sup>

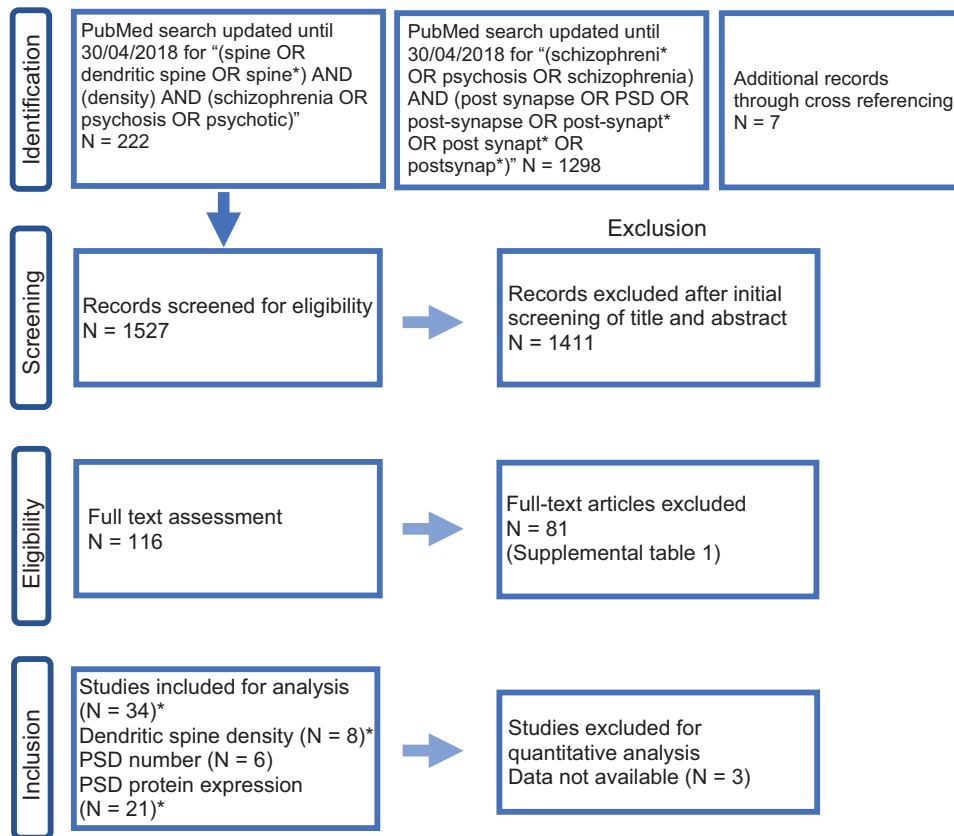
We identified 34 individual studies assessing structural measurements of postsynaptic elements: DSD (8), PSD number (6), and PSD protein expression (21) for qualitative analysis. One study measured both DSD and PSD protein expression.<sup>50</sup> These studies considered 12 different brain regions (figure 1E) and a variety of PSD proteins (figure 1F). Replication studies, analyzing the exact same measurement in the same region in at least 3 separate cohorts, were scarce. Only PSD95 measurements were replicated in the hippocampus,<sup>50-53</sup> anterior cingulate cortex (ACC),<sup>54-56</sup> and dorsolateral prefrontal cortex.<sup>51,54,55,57,58</sup> This limits the opportunity to perform separate analyses of specific brain regions. Therefore, we assessed all data together to then further explore where possible sources of heterogeneity in subanalyses. An overview of included studies and extracted data can be found in supplementary table 2<sup>12,49,50,52-54,56-78</sup>.

### Qualitative Analysis

We performed a quality assessment for all 34 studies assessing methodology, study design, and reporting (supplementary table 3). Although postmortem studies are labor intensive and involve many premortem and postmortem confounders, our assessment showed that in general the included studies were of good quality. Most studies reported the demographics in full, described their applied methods extensively, performed matching, and controlled for important confounders (age/PMI/sex). However, 16 studies did not report on neuropathological examinations. As changes in synapse number have been described in a number of neurodegenerative diseases,<sup>79</sup> neurologic comorbidity could be an important confounder. Moreover, we found that 16 studies did not report on blinding the experiment and 6 studies did not report on the method of SCZ diagnosis.

### Primary Analysis: Association of Postsynaptic Element Density in SCZ Postmortem Brain

We performed a random-effects meta-analysis on 31 separate studies, including all brain regions and all 3



**Fig. 2.** PRISMA flowchart. Diagram of the systematic search strategy. \*One paper reported data on both dendritic spine density and postsynaptic density protein expression.

study categories (comprising 98 individual datapoints). To prevent overrepresentation of studies including multiple measurements, estimated ES within each study were nested. Meta-analysis of the nested data showed that the density of postsynaptic elements is lower in SCZ patients than in control subjects (figure 3; ES:  $-0.33$ ; 95% CI:  $-0.60$  to  $-0.05$ ;  $P = .020$ ). A similar result was obtained performing the analysis with unnested data (supplementary figure 1; ES:  $-0.22$ ; 95% CI:  $-0.37$  to  $-0.07$ ;  $P = .004$ ).

We detected high between-study heterogeneity ( $I^2$ : 78.39%;  $Q$ : 138.90;  $P < .001$ ). Sensitivity analysis, excluding studies with a residual z-score  $\pm 1.96^{16}$ , showed no significant but trend level decrease in postsynaptic elements (supplementary figure 2; ES:  $-0.24$ ; 95% CI:  $-0.48$  to  $-0.003$ ;  $P = .053$ ). Although decreased, heterogeneity remained moderate ( $I^2$ : 70.59%;  $Q$ : 98.61;  $P < .001$ ).

Publication bias was assessed based on visual inspection of the funnel plot and Egger's regression test. No asymmetry was observed by visual inspection, which was confirmed by Egger's regression test ( $P = .42$ ) (supplementary figure 3).

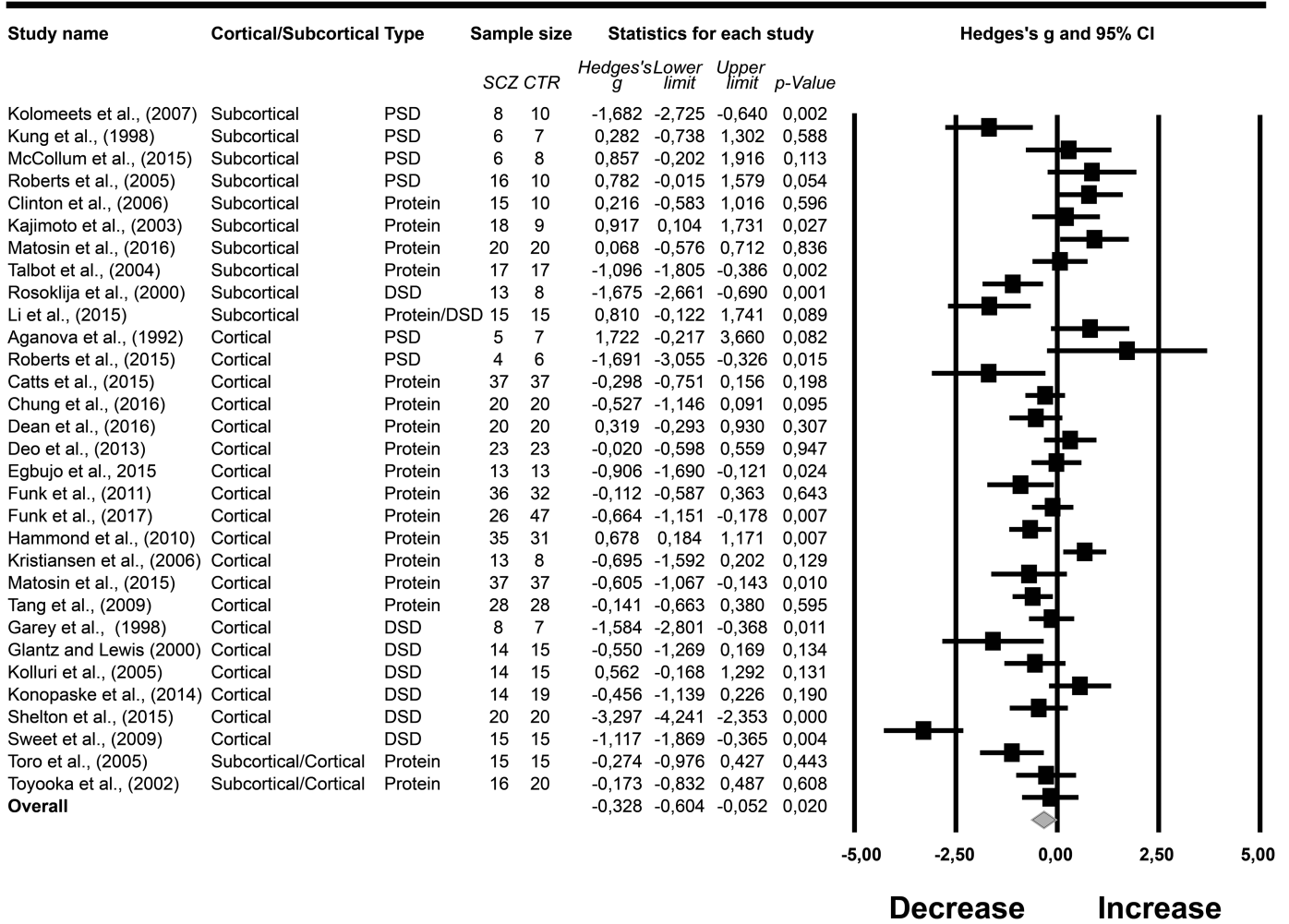
We performed meta-regression analyses to check potential continuous (age, sex distribution, and PMI) and categorical (brain bank) confounder variables. Age, sex,

PMI, and brain bank showed no moderating effects on outcome measurements (supplementary figure 4;  $P > .05$ ).

#### Subgroup Analysis: Stratified by Brain Region and Study Category

To assess possible sources of variation, we performed subgroup analyses. Data from the same study were included in both analyses when data were reported separately for each group.<sup>50-52,80</sup> First, we separated cortical and subcortical studies. Subgroup analyses revealed a significant decrease in density of postsynaptic elements in cortical tissues (figure 4A; ES:  $-0.44$ ; 95% CI:  $-0.76$  to  $-0.12$ ;  $P = .008$ ) but no change in subcortical tissues (figure 4A; ES:  $-0.11$ ; 95% CI:  $-0.54$  to  $0.35$ ;  $P = .671$ ). However, the  $Q$ -test-based ANOVA for subgroup differences indicated no significant difference between the 2 groups ( $Q_{\text{between}} = 1.50$ ;  $P = .221$ ). No publication bias (supplementary figure 5) or confounding effects of age, sex, PMI, and brain bank (supplementary figure 6) were found. High between-study heterogeneity remained in both cortical ( $I^2$ : 77.98%;  $Q$ : 90.82;  $P < .001$ ) and subcortical ( $I^2$ : 76.18%;  $Q$ : 46.17;  $P < .001$ ) studies.

A subgroup analysis was also performed separating the 3 study categories (DSD, PSD number, and PSD



**Fig. 3.** Forest plot of primary meta-analysis on density of postsynaptic elements in schizophrenia (SCZ). The pooled effect size of all studies on postsynaptic elements indicates that the density of postsynaptic elements is decreased in SCZ ( $P < .05$ ). PSD, postsynaptic density number; Protein, PSD protein expression level; DSD, dendritic spine density.

protein expression). We found a significant decrease in DSD (figure 4B; ES:  $-0.81$ ; 95% CI:  $-1.37$  to  $-0.26$ ;  $P = .004$ ) and no difference for PSD protein expression (figure 4B; ES:  $-0.17$ ; 95% CI:  $-0.51$  to  $0.16$ ;  $P = .320$ ) or PSD number (figure 4B; ES:  $-0.01$ ; 95% CI:  $-0.72$  to  $0.70$ ;  $P = .98$ ). However, no difference between groups was detected as shown by the  $Q$ -test-based ANOVA ( $Q_{\text{between}} = 4.45$ ;  $P = .108$ ). No publication bias (supplementary figure 7) or confounding effects of age, sex, PMI, and brain bank (supplementary figure 8) were found. Moderate to high heterogeneity was observed in all study categories; DSD ( $P$ : 88.72%;  $Q$ : 62.07;  $P < .001$ ), synapse density ( $P$ : 80.31%;  $Q$ : 25.39;  $P < .001$ ) and PSD protein expression ( $P$ : 61.44%;  $Q$ : 44.09;  $P < .001$ ).

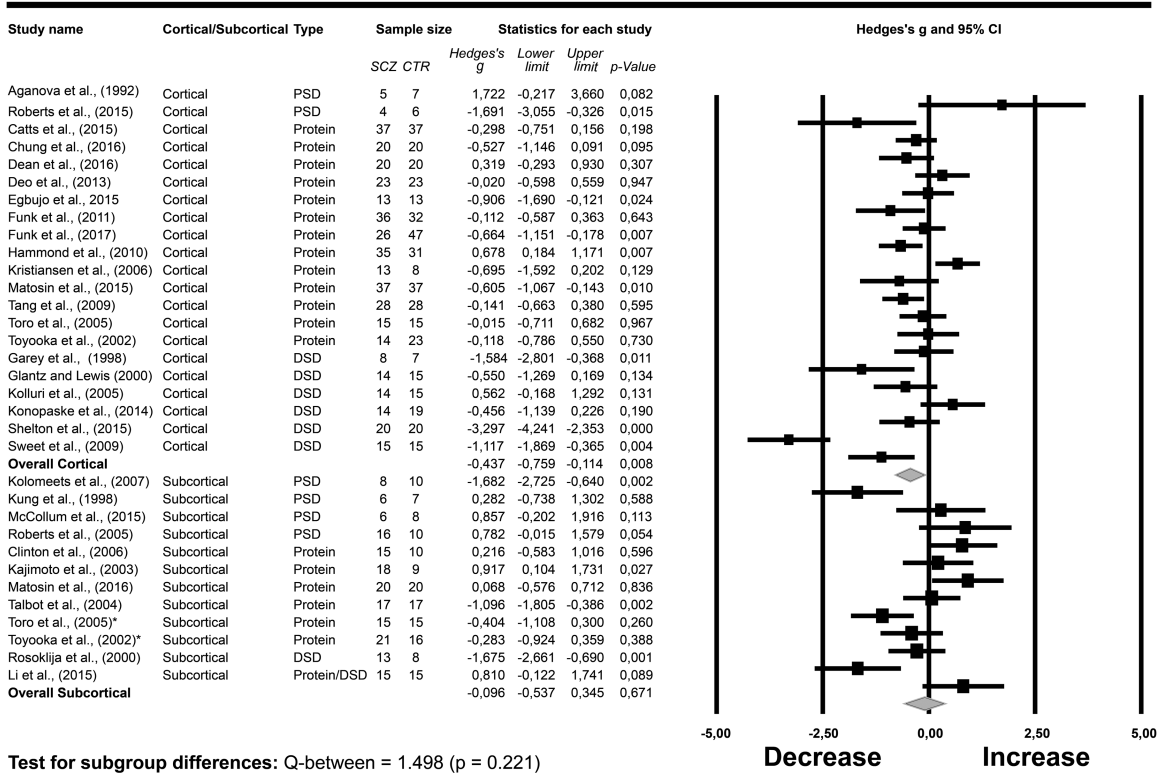
Surprisingly, we identified a study reporting significant opposite effect directions in the expression of PSD proteins: showing an upregulation for Homer1a and Preso and downregulation for PSD95 and Homer1b/c in the hippocampus.<sup>53</sup> This was also the case at a nonsignificant

level in other studies.<sup>49-51,54,61-63</sup> To visualize the variation in expression of different PSD proteins in SCZ post-mortem tissue, we generated a forest plot with unnested data of all PSD protein expression studies (supplementary figure 9).

*Exploratory Subanalyses: Specific Brain Areas*

Lastly, we performed exploratory subgroup analyses when 5 or more studies were performed on the same brain area. These analyses showed a significant decrease of postsynaptic elements in the prefrontal cortex (figure 5A; ES:  $-0.27$ ; 95% CI:  $-0.53$  to  $-0.01$ ;  $P = .043$ ) and cortical layer 3 (figure 5B; ES:  $-1.39$ ; 95% CI:  $-2.24$  to  $-0.54$ ;  $P = .001$ ). No change was found in the ACC (figure 5C; ES:  $-0.25$ ; 95% CI:  $-0.97$  to  $0.47$ ;  $P = .50$ ) and the hippocampus (figure 5D; ES:  $-0.57$ ; 95% CI:  $-1.17$  to  $0.02$ ;  $P = .059$ ). A graphical representation of these results is depicted in figure 6. Heterogeneity in these analyses was

### A. Stratified by brain region



### B. Stratified by study category

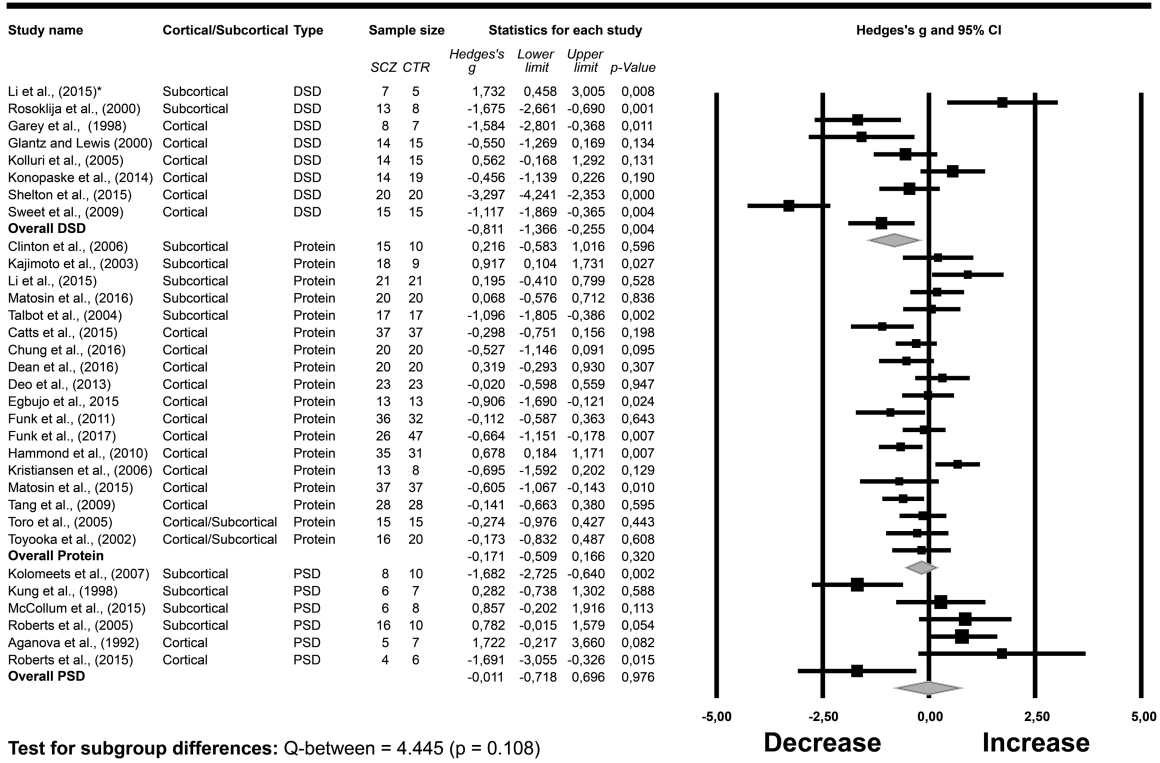
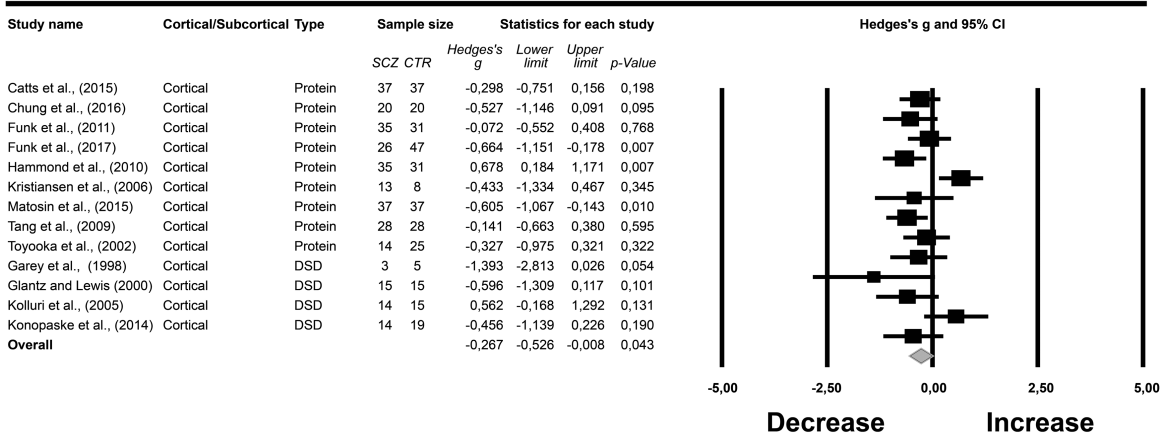
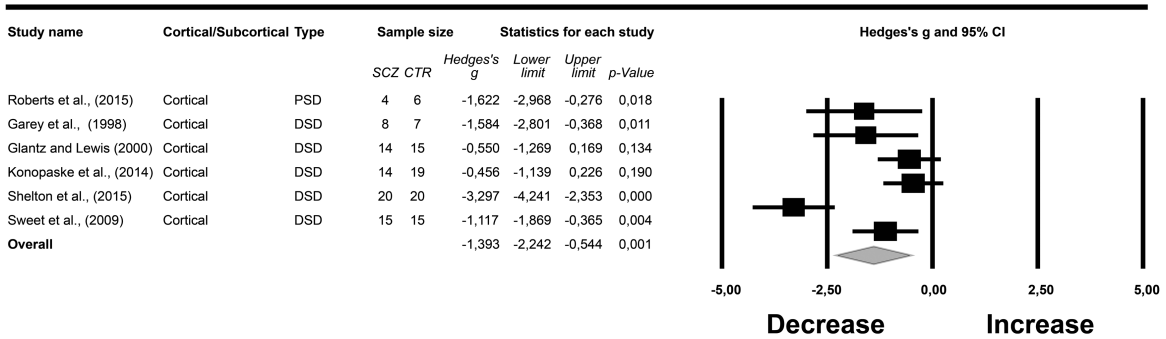


Fig. 4. Forest plots of subgroup meta-analyses on density of postsynaptic elements in schizophrenia (SCZ). Subgroup meta-analyses for postsynaptic density (PSD) in SCZ stratified per (A) brain region (cortical/subcortical) and (B) study category. The pooled effect size of studies on the density of postsynaptic elements in cortical tissues is decreased in SCZ ( $P < .05$ ) but not significantly changed in studies on subcortical tissues ( $P > .05$ ). PSD, PSD number; Protein, PSD protein expression level; DSD, dendritic spine density.

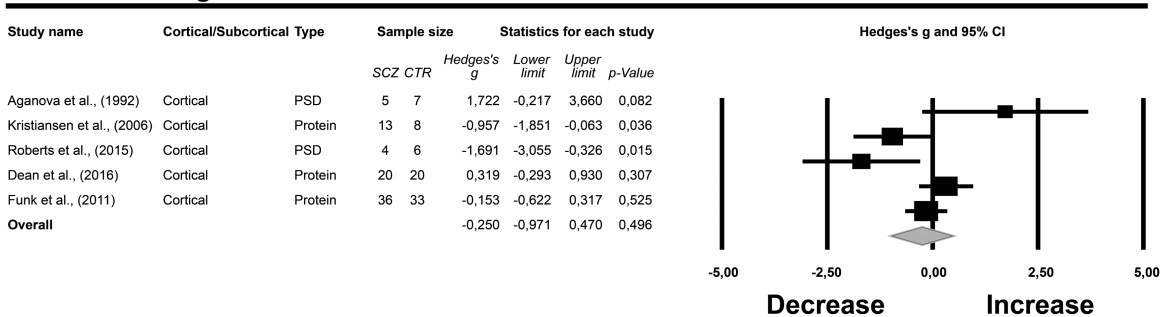
### A. Prefrontal Cortex



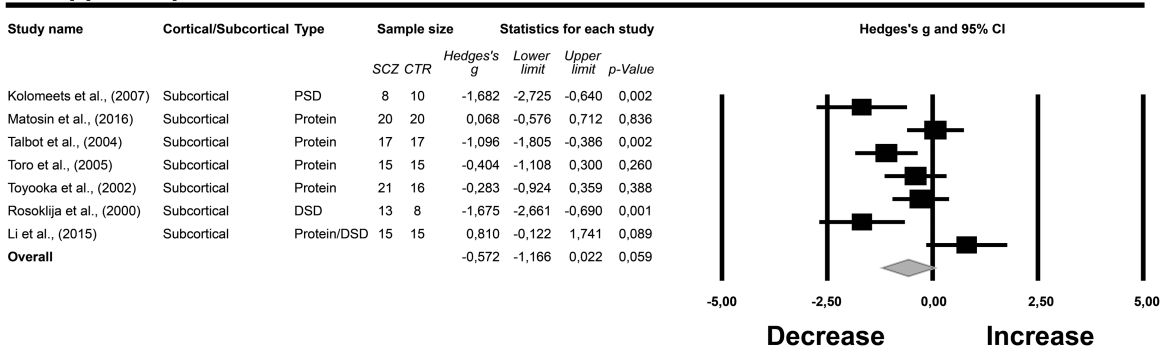
### B. Cortical Layer 3



### C. Anterior Cingulate Cortex



### D. Hippocampus



**Fig. 5.** Forest plots of brain area specific exploratory subanalyses. Exploratory subgroup meta-analyses for postsynaptic elements in schizophrenia (SCZ) in the (A) prefrontal cortex (PFC), (B) cortical layer 3, (C) anterior cingulate cortex (ACC), and (D) hippocampus. The pooled effect sizes of studies on the density of postsynaptic elements in the PFC and layer 3 are significantly decreased ( $P < .05$ ) but not changed in studies on the ACC and hippocampus ( $P > .05$ ). PSD, postsynaptic density number; Protein, PSD protein expression level; DSD, dendritic spine density.



moderate in the PFC ( $I^2$ : 58.42%;  $Q$ : 28.86;  $P$  = .004) and high in cortical layer 3 ( $I^2$ : 81.79%;  $Q$ : 27.46;  $P$  < .001), the ACC ( $I^2$ : 71.14%;  $Q$ : 13.86;  $P$  = .008), and hippocampus ( $I^2$ : 75.21%;  $Q$ : 24.20;  $P$  < .001).

## Discussion

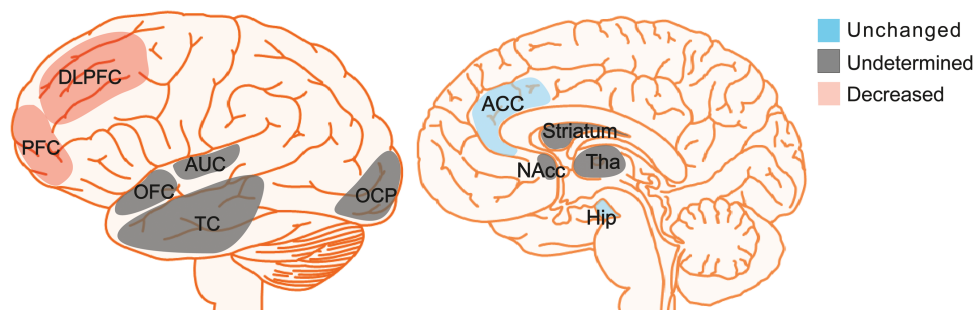
In this study, we quantitatively investigated 3 outcome measures reflecting the number of postsynaptic elements in SCZ postmortem brain tissue: DSD, PSD number, and PSD protein expression. Our meta-analysis showed a significant decrease in density of postsynaptic elements in SCZ patients compared to healthy controls. However, sensitivity analyses showed high heterogeneity, suggesting the presence of subgroups. No evidence was found for publication bias or confounding factors (age, PMI, sex, and brain bank). With our meta-analysis, we quantitatively assessed, to our knowledge, the largest sample size to date on structural abnormalities of postsynaptic elements in postmortem brain tissue of SCZ patients, providing an extensive overview of the current literature on this topic.

At the same time, we recognize that several of the included studies were performed on sample populations from the same brain bank or cohort.<sup>12,69,70,73,74,76,81</sup> It was not feasible to determine which parts of the samples were overlapping to compute separate ES. This could result in an overrepresentation of specific populations in our meta-analysis. Furthermore, our research design provided evidence that alterations in postsynaptic elements were not due to age, sex, or PMI of the studied subjects. However, given the limited availability of data, several potential confounding factors such as suicide rate, severity of symptoms, and antipsychotic use could not be considered. Confounding by these factors is unavoidable in SCZ postmortem research and should, therefore, be addressed in future analyses. In particular, the use of antipsychotics has been suggested to influence synapse density.<sup>82,83</sup> Although some studies (10) did not/could not correct for medication use,<sup>49,51,54,58,69,71,73,74,76,78</sup> most studies included in our meta-analyses (18) found no association between medication use and the outcome measurement.<sup>12,48,50,52,57,61–68,70,72,75,77</sup>

Thus, while our study shows a decrease in density of postsynaptic elements in SCZ, future research will need to address the contribution of these confounding factors.

High heterogeneity was observed among included studies in the primary analysis. Although this is common in meta-analyses on preclinical data,<sup>40</sup> it should be considered and explored. A priori, we defined brain region and study category as potential sources of heterogeneity. Our subgroup and exploratory subanalyses showed a significant decrease of postsynaptic elements in cortical regions, specifically in the PFC and cortical layer 3. We did not observe this effect in subgroup analysis for subcortical regions or in analyses of the ACC and hippocampus. Although this suggests that the effect is most pronounced in cortical tissues, subgroup differences between cortical and subcortical studies were not statistically significant. Regional heterogeneity of postsynaptic element deficits in SCZ has been hypothesized before.<sup>13,16</sup> An earlier study showed that spine density was decreased in cortical layer 3 but not in layer 5/6 of the same cohort.<sup>69</sup> Studies of the basal ganglia show an opposite effect, with an increase of PSD number.<sup>74,77</sup> These changes also seem to be specific to subregions as increases are exclusively found in the core compartment of the nucleus accumbens<sup>74</sup> and in the caudate but not the putamen of the striatum.<sup>77</sup> It should be considered that we were unable to perform meta-analysis for each brain region separately as most are underrepresented in our data set. Strikingly, electron microscopy studies are almost exclusively performed on subcortical tissues, while most dendritic spine studies are performed selectively in cortical layer 3. Other cortical layers were researched in 2 separate studies.<sup>69,73</sup> Systematic analysis of different brain regions and replication studies with large cohorts, recently shown feasible for transcriptomic studies,<sup>84,85</sup> are necessary to compare specific brain areas to fully identify sources of heterogeneity.

Understanding local heterogeneity could also help determine the neuronal populations most vulnerable to pathology in SCZ. Our study has focused primarily on excitatory synapses. Dendritic spines form the primary source of excitatory input,<sup>24–26</sup> and the structural proteins of the PSD in our analysis are almost exclusively found



**Fig. 6.** Schematic representation of changes in postsynaptic elements. The schematic represents changes in postsynaptic elements in SCZ for brain regions tested with meta-analyses (unchanged or decreased) and shows which brain regions could not be tested (undetermined).

in excitatory synapses.<sup>32,86</sup> Furthermore, with exception of one study,<sup>87</sup> most electron microscopy studies show that effects are specific for excitatory (asymmetric and axospinous) synapses.<sup>43,73,74,77</sup> Although impaired inhibition also has been hypothesized to affect cognition in SCZ, few structural postmortem studies have been performed to assess this.<sup>88</sup>

Our subgroup analysis identified no significant difference between the 3 study categories, DSD, synapse density, or PSD protein expression. However, we identified a significant decrease in DSD, suggesting that the effect is most pronounced in dendritic spines. Some electron microscopy studies, indeed, show a specific decrease of axospinous synapses in SCZ.<sup>73,75</sup> An alternative explanation for these findings could be the brain regions represented in each category. Subcortical studies are overrepresented in the category of PSD number and are less prevalent in DSD studies.

Our subgroup meta-analysis showed no significant difference in PSD protein expression in SCZ. Unexpectedly, some studies show opposite regulation of different individual PSD proteins, a phenomenon masked in our analysis because we nested the data. It suggests that the expression level of some PSD proteins is actively regulated in SCZ and is not only a consequence of the number of synapses.

Possible mechanisms explaining the decrease in density of postsynaptic elements found in our meta-analysis include deficits in synapse formation, maintenance, or elimination. Defects in synapse formation are suggested by studies identifying SCZ risk genes encoding for PSD scaffolding proteins like *DISC1*, *SHANK*, and *HOMER*.<sup>11</sup> Altered synapse stabilization is implicated by a study showing that especially smaller, transient dendritic spines are decreased in SCZ.<sup>44</sup> SCZ risk genes like *CACNB2* and *CACNB4*<sup>89</sup> could affect local calcium transients at dendritic spines, necessary for their stabilization.<sup>44,90,91</sup> Alternatively, noncell autonomous involvement of glia (microglia and astrocytes) might play a role. Studies have shown altered secretion of astrocytic gliotransmitters, necessary for synapse stability.<sup>92,93</sup> Furthermore, increased glial pruning of synapses is suggested by a recent in vitro study<sup>94</sup> and because of the high association between complement 4 genes in the MHC locus and risk of developing SCZ.<sup>89,95</sup>

A recent elaborate transcriptomic study from the PsychENCODE consortium, using cortical brain tissue, could provide insight in postsynaptic element dysfunction in SCZ.<sup>85</sup> Most genes coding for proteins assessed in our meta-analyses were not differentially expressed (supplementary table 4). However, many novel SCZ risk genes, related to synaptic or glial function, were suggested; for example, the kinase *DCLK3*, which enhances dendritic remodeling and synapse maturation and,<sup>85,96</sup> also, the astrocytic glutamate transporters *SLC1A3* and *SLC1A2*, which dysfunction could affect astrocyte-synapse

interaction at excitatory synapses.<sup>85,97</sup> More SCZ risk genes were identified within the modules of PSD/trans-synaptic signaling, astrocytes, and microglia that need to be further explored in future studies assessing the relation to synapse dysfunction.

In general, the observed decrease of postsynaptic elements in the cortex, and layer 3 specifically, could be related to the clinical phenotype of SCZ. Cortical layer 3 contains pyramidal neurons, important for corticocortical projections.<sup>98</sup> These projections are required for higher cognitive functions, like working memory, which are affected in SCZ.<sup>88,99</sup> A decrease in excitatory synapses is predicted to result in a reduced excitatory drive, possibly resulting in hypoactivity of layer 3 neurons. Previously, decreases in spine density were shown to be associated with alterations in connectome architecture as measured with diffusion tensor imaging.<sup>100</sup> Therefore, microscale deficits in synapse structure and function could influence brain connectivity at macroscale, potentially underlying the symptoms observed in SCZ. Altogether, the overall decrease in postsynaptic elements in the cortex also provides a specific cellular hallmark for translational research in SCZ that could be studied in human cell culture systems, brain organoid models, and animal studies. However, study approaches extending histological analyses to integrate cellular phenotypes with proteomic, transcriptomic, genomic, and clinical data in large cohorts are imperative for translational research.

Furthermore, this phenotype also provides a possible target for diagnostics and novel therapeutics. Interestingly, several positron-emission tomography (PET) tracers visualizing presynaptic elements in vivo have been developed,<sup>101</sup> providing means to analyze synapse density in the living human brain. Currently, PET tracers for postsynaptic elements are targeted toward receptor proteins, like the NMDA and dopamine receptor, which are suspected to be actively regulated in SCZ. The development of intracellular PET tracers for postsynaptic scaffolding proteins would contribute to the analysis of postsynaptic element dynamics during disease states and could provide a biological outcome measurement for diagnostic purposes. Eventually, strategies that target postsynaptic elements, for instance stabilizing PSD integrity, could present a novel therapeutic approach in the treatment of SCZ.

## Supplementary Material

Supplementary data are available at *Schizophrenia Bulletin* online.

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