Supplementary Online Content


eMethods
eResults
eReferences
eFigure 1. Rich club connectivity density effects at different rich club levels. Rich club regions as described throughout the main report were set at a rich club level of $k>15$. eFigure 1 shows the influence on the reported rich club connectivity density effects for different group-based rich club levels. eFigure 1 shows for group rich club levels $k>11$ to $k>22$ the average rich club connectivity density for the group of controls (dark green), group of patients (light green), the group differences (blue line), the statistical p-value of this difference (permutation testing, 10,000 permutations) and the number of rich club members, per rich club level (varying from 24 to 2). Significant reduced rich club density in patients was found at a range of levels, from $k>14$ to $k>19$ (all $p < 0.01$, 10,000 permutations), indicating that rich club differences are also present at other group based on basis of the group averaged cortical network rich club levels.
eFigure 2. Connectivity density of rich club, feeder and local connections with the rich club defined on an individual basis. In the main analysis, the brain’s rich club was a priori defined on basis of the group-averaged network at a rich club level of $k>15$. However, as an alternative approach, differences in connectivity density between patients and controls was also examined with the rich club selected on an individual basis, selecting the rich club within each individual data set separately as (1) the top 12% highest (degree based) ranking nodes (panel A) and/or (2) nodes with a degree higher than 1.25 standard deviations of the average degree of the network (panel B). Consistent with the findings reported in the main text (i.e. rich club selection on a group basis), both individual approaches showed significant reduced rich club density in patients as compared to controls (A: $p = 0.0018$, B: $p = 0.0390$ indicated by *, 10,000 permutations), while the effect was less pronounced or not present in the feeder and local connections (A: feeder, $p = 0.113$, B: feeder $p = 0.049$ | A: local, $p = 0.782$, B: local $p = 0.245$, 10,000 permutations).
eFigure 3. Relationship between regional volume and connectivity (A). eFigure 3a shows the relationship between nodal degree (binary) and regional volume, confirming a relationship between volume of a node and its binary degree. (B) eFigure 3b shows the relationship between nodal strength (sum of streamlines) and regional volume, showing a clear relationship between nodal volume and streamline density. (C) eFigure 3c shows the relationship between nodal strength (streamlines density) and regional volume, showing that dividing off regional volume corrects for the influence of regional volume on connectivity strength. eFigure 3D shows, over all edges in the network, the association between connectivity strength (streamline count) and sum of the volume of the target and source nodes, showing no clear relationship. eFigure 3E shows the same plot as figure 3D, but now with streamline density. Panels D and E illustrate that two high volume regions do not show a high streamline connectivity and/or high connectivity density per se.
This supplementary material has been provided by the authors to give readers additional information about their work.
Participants

Two data sets were included in this study: (1) a principal data set involving 48 patients and 45 healthy controls collected at 3 Tesla from 2008 and 2010 and (2) an independent replication data set including 41 patients and 51 matched controls acquired on 1.5 Tesla MRI between 2007 and 2009. Data sets of the principal and replication data set did not overlap with data from previous network studies conducted by our group.¹

Principal data set. Forty-eight patients with schizophrenia and 45 healthy controls participated, referred to as the principal data set of the study. Demographics of the principal data set are listed in Table 1 of the main text.

Replication data set. A second group of 41 patients and 51 controls was included, serving as a replication data set to replicate possible results found in the principal group. Structural DTI data was available from all 41 patients and from 51 controls; both structural (DTI) and functional (resting-state fMRI) data was available in 39 patients and 35 controls. Demographics of the replication set are listed in Table 1 of the main text.

All participants of the principal and replication data set provided written informed consent as approved by the medical ethics committee for research in humans (METC) of the University Medical Center Utrecht, The Netherlands. The study was carried out according to the directives of the Declaration of Helsinki (amendment of Edinburgh, 2000). Patients and controls were matched (per set) for age and gender. All participants underwent psychiatric assessment procedures using the Comprehensive Assessment of Symptoms and History (CASH). Healthy participants were recruited by means of local newspaper advertisements and did not have a first-degree relative with any mental illness or a second-degree family member with a psychotic disorder. Diagnostic consensus of patients was achieved in the presence of a psychiatrist, meeting DSM-IV criteria for
schizophrenia. At time of scanning, all patients were receiving typical or atypical antipsychotic medication.

**Acquisition**

*Principal data set.* The principal data set was acquired on a 3 Tesla Philips Achieva clinical scanner at the University Medical Center Utrecht, using an eight-element SENSE receiver head-coil.

Participants underwent a 45 minute scanning session, in which Diffusion Tensor Imaging (DTI) and resting-state fMRI data was acquired.\(^2\)\(^-\)\(^4\)  *Diffusion Tensor Imaging.* Within each scanning session, two sets of DTI images each consisting of 30 diffusion weighted volumes -all with a b-factor = 1000 s/mm\(^2\), non-collinear directions\(^5\)\(^-\)\(^6\) - and 5 diffusion un-weighted B=0 volumes -b-factor = 0 s/mm\(^2\)- were acquired (*parameters*: parallel imaging SENSE p-reduction 3; TR/TE = 7035/68 ms, 2 mm isotropic voxel size, 75 slices, b=1000 s/mm\(^2\), second set with reversed k-space read-out).

*Resting-state fMRI.* Resting-state Blood Oxygenation Level Dependent (BOLD) signals were recorded during a period of 8 minutes (*parameters*: 3D PRESTOSENSE, TR/TE 22/32 ms using shifted echo, flip-angle 9 degrees; p/s-reduction 2/2; dynamic scan time 502 ms, 4 mm isotropic voxel size, 32 slices covering whole brain). The short volume acquisition time of 500 ms was used to minimizing back-folding of effects of higher frequencies (i.e. cardiac and respiratory oscillations) into the lower frequencies of interest (0.01 - 0.1 Hz).\(^7\)  *Anatomical T1.* In addition, a T1-weighted image was acquired for anatomical reference. (*parameters*: 3D FFE using parallel imaging; TR/TE 10ms/4.6ms; FOV 240x240mm, 200 slices, 0.75 mm isotropic voxel size).

*Replication data set.* The replication data set was acquired on a 1.5 Tesla Philips Achieva clinical scanner at the University Medical Center Utrecht, using a six-element SENSE receiver head-coil. Participants underwent a 35 minutes scanning session, in which both validated Diffusion Tensor Imaging (DTI) and resting-state fMRI data were acquired.\(^1\)\(^4\)\(^8\)  *Diffusion Tensor Imaging.* In each scanning session, two consecutive sets of DTI images each consisting of 32 diffusion-weighted
volumes with different non-collinear diffusion directions -all with a b-factor = 1000 s/mm²- and 8 diffusion unweighted volumes -with a b-factor = 0 s/mm²- were acquired (parameters: DTI TR/TE = 9822/88 ms, parallel imaging factor: 2.5; 60 slices each with 2.5 mm isotropic voxels covering the whole brain, no slice gap, FOV 240 mm, 128x128 acquisition matrix). Resting-state fmRI. BOLD time-series were recorded for a period of 9 minutes using a 3D-PRESTO sequence (parameters: TR/TE 21.1/31.1 ms; dynamic scan time 538 msec, 4 mm isotropic voxel size, 64x64 acquisition matrix, 36 slices covering the whole brain). Anatomical T1. A T1 weighted image was acquired for anatomical reference (parameters: 3D FFE pulse sequence, TR/TE = 30 ms / 4.6 ms, FOV 256 x 256 mm, voxel size 1 x 1 x 1.2 mm, 160-180 contiguous slices).

Data preprocessing

Preprocessing of the principal data set

Data processing steps are illustrated in Figure 1 of the main text. T1 preprocessing and cortical parcellation. Based on the T1 image, the gray matter cortex was parcellated into distinct regions using the Freesurfer software suite (V5.0)⁹ (Figure 1a). This included the automated segmentation of grey and white matter brain tissue, followed by a 3D reconstruction of the cortical mantle and automatic parcellation of the reconstructed mantle into 68 distinct cortical regions. In addition, to the 68 cortical regions, 14 subcortical regions were segmented, including left and right hemispheric hippocampus, amygdala, accumbens, caudate, putamen, pallidum and thalamus.

Diffusion Tensor Imaging. First, the two sets of 30 DTI images and 2 averaged B=0 images (averaged over the 5 B=0 images) were corrected for small head movements by re-aligning all scans to the B=0 images.¹⁰ Susceptibility distortions (often reported in high-field DTI acquisitions) were corrected by computing a field distortion map based on the two unweighted B0 images (based on the information that they were acquired with an opposite k-space read-out direction), which was in turn applied to the two sets of weighted DTI scans.¹¹ This resulted in a corrected set of 30 weighted diffusion directions and one averaged B=0 image.¹¹ Second, for each voxel, the diffusion profile
was fitted to a tensor using a robust tensor fit method based on an M-estimator,\textsuperscript{12} and the preferred diffusion direction was selected as the principal eigenvector of the eigenvalue decomposition of the tensor. Third, within each voxel the fractional anisotropy (FA) was computed (Beaulieu and Allen, 1994; Basser and Pierpaoli, 1996), with higher FA values indicating a higher level of directionally restricted diffusion of water molecules. Finally, for each individual DTI data set, white matter pathways were reconstructed, referred to as fibers or tracts, using streamline tractography. For this, the \textit{Fiber Assignment by Continuous Tracking} (FACT) algorithm was used\textsuperscript{13,14} (Figure 1b). For each voxel within the brain mask, 8 seeds were started, following the main diffusion direction from voxel to voxel, and a streamline was terminated when it reached a voxel with a FA value lower than 0.1 (reflecting low levels of preferred diffusion, often grey matter voxels), when the streamline exceeded the brain mask (i.e. grey and white matter voxels), or when the trajectory of the streamline made a turn sharper than 45 degrees. Streamlines longer than 30 mm were considered for further analysis.

\textit{Resting-state fMRI.} First, resting-state time-series were realigned and co-registered with the T1 image to ensure overlap with the cortical parcellation maps. Next, time series were de-trended (i.e. linear trends and first order drifts were removed), corrected for global effects (regressing out the white matter, ventricle, and global mean signals, as well as 6 motion parameters) and band-pass filtered (0.01 - 0.1 Hz) (Figure 1c).

\textit{Preprocessing of replication data set}

\textit{T1 preprocessing and cortical parcellation.} Processing of the T1 images of the replication set was identical to the analysis performed on the 3T data of the principal data set (see above).

\textit{Diffusion Tensor Imaging.} First, the two DTI sets were realigned, corrected for eddy currents\textsuperscript{10} and concatenated. Second, the diffusion profile in each voxel was fitted to a tensor using a robust tensor fit method,\textsuperscript{12} using the weighted images and the averaged B=0 image (resulting from averaging over the 5 B=0 images). Next, the main diffusion direction in each voxel was selected as the
principal eigenvector from the eigenvalue decomposition of the fitted tensor. FA values were computed and whole brain streamline tractography was performed (following the same procedure as described in the main set).

Resting-state fMRI. Preprocessing of the resting-state time-series of the replication data set included the same steps as performed for the preprocessing of the principal data set.

Connectivity analysis

For each individual subject, using the collection of reconstructed fiber tracts and the filtered resting-state time-series, structural (SC) and functional connectivity (FC) networks were created. All processing steps were identical for the principal data set and the replication data set.

Construction of structural brain networks

For each individual data set a structural brain network was created by combining the collection of reconstructed fiber tracts with the individual parcellation map (Figure 1b). A network consists of nodes and connections that can be mathematically expressed as a graph $G=(V,E)$, with $V$ the collection of nodes and $E$ the collection of edges between the nodes. Within this study, (for both the functional and structural data) two networks were created: (1) a cortical network consisting of 68 cortical regions (referred to as the cortical network) and (2) a brain network consisting of 68 cortical and 14 subcortical regions (referred to as whole brain network). Networks were constructed as follows. First, parcellated brain regions were taken to represent the nodes in both the structural and functional analysis. Two network nodes $i$ and $j$ were defined as being connected when a set of fibers was found from the total collection of reconstructed streamlines that interconnected them (Figure 1-I). If no streamlines were found between $i$ and $j$, regions $i$ and $j$ were taken to be unconnected. Information on the existence (and non-existence) of a connection between node $i$ and $j$ was collected in the connection matrix, with each cell $(i,j)$ of the matrix representing the number of streamlines that interconnected $i$ and $j$. As such, each individual weighted connectivity matrix $M$
reflected a matrix representation of an individual brain network, with the number of reconstructed streamlines representing the strength of the connections between the nodes in $G$. In addition to analyzing weighted networks based on absolute streamline count, an analysis was performed in which the weights of the connectivity matrix $M$ (i.e. streamline count) were expressed as streamline densities, computed as the number of streamlines divided by the individual volumes of the two interconnected regions of interest (i.e. nodes of the graph).\textsuperscript{15,16}

\textit{Construction of functional brain networks}

Next, using the band-pass filtered resting-state fMRI time-series, for each individual data set, the level of functional connectivity (FC) between all nodes of the network was computed (Figure 1c). The nodes of the functional network were taken in a similar fashion as the nodes in the structural network (i.e., the 68/82 parcellated cortical/sub-cortical regions) to enable direct comparison between the two types of networks. First, for node $i$ the fMRI voxels that overlapped with region $i$ were selected and the time-series of the selected voxels were averaged. Second, the averaged regional time series of node $i$ and $j$ were correlated, taken as the level of functional coupling between region $i$ and $j$ and their level of correlation was included in the functional connection matrix, with each cell $(i,j)$ of the matrix representing the level of functional connectivity between node $i$ and $j$ in the network. In register with the individual structural network, for each individual data set, this resulted in a weighted $N \times N$ matrix reflecting the functional connections between the $N=68/82$ brain regions (Figure 1c).

\textit{Rich club organization.} The focus of our study was the investigation of rich club organization of the brain’s network in patients and controls. Rich club organization of the human connectome was described in detail in a previous paper.\textsuperscript{16} The so-called rich club phenomenon in networks is said to be present when the highly connected (high-degree) hubs of a network are more densely connected among themselves than predicted on the basis of their high degree alone.\textsuperscript{17} To identify rich club
organization, the number of connections among high-degree nodes is compared to the number of
corresponding connections that would occur by chance alone. In addition, a weighted version of the rich club
behavior incorporates the weights of the edges in the network, examining the level of density
between the subset of selected nodes in the network. For all individual structural networks the
weighted-rich club coefficient $\Phi_w(k)$ was computed as follows. First all non-zero connections of
the examined number-of-streamlines-weighted network were ranked in respect to their weight,
resulting in a vector $W_{\text{ranked}}$. Second, within the connectivity matrix $M$, for each value of degree $k$,
the sub-graph of nodes with a degree larger than $k$ was selected (with $k$ defined as the number of
each node’s binary connections). Third, the number of links $E_{>k}$ present between the members of the
subset was determined and the sum of their collective weight $W_{>k}$ was computed. Fourth, the
maximal level of connectivity between the top number $E_{>k}$ of connections in the network was
determined, again computed as the sum of the weights. Finally, the weighted rich club parameter
$\Phi_w(k)$ was computed as the ratio between $W_{>k}$ and this sum of the strongest number of links $E_{>k}$ in
the total network. Formally, $\Phi_w(k)$ is given by.

$$
\Phi_w(k) = \frac{W_{>k}}{\sum_{E_{>k}} W_{\text{ranked}}}
$$

Note that the rich club coefficient as defined in equation (1) is invariant to a uniform decrease of
connectivity of the network. A uniform decrease of all weights of all connections will change the
nominator and denominator of equation (1) at equal proportion, resulting in an identical $\Phi_w(k)$
value. In contrast, a non-uniform change (meaning that only a subset of connections is changed, or
in the case that some connections are changed more than others) can have a differentiating effect on $\Phi_w(k)$.

The weighted version of the rich club coefficient as introduced by Opsahl et al. can be
interpreted as a mixture of a binary and weighted metric, examining whether a group of nodes has
1) an above chance level of edges (i.e. binary connections) between them, and 2) whether these
connections have a proportional high level of weight (i.e. strength) as compared to the other
connections of the network and as compared to a random null model (see below).

Normalization. Random networks also show an increasing function of $\Phi^w(k)$ related to the fact that nodes with a higher degree display a higher probability of being interconnected by chance alone. Hence $\Phi^w(k)$ is typically normalized relative to a (set of) comparable random network(s), to determine the extent to which empirically observed connection density between rich club nodes exceeds that predicted by the random null model, driven by node degree alone. Comparison of $\Phi^w(k)$ to $\Phi_{\text{random}}(k)$ obtained from a population of random networks, resulted in a normalized rich club coefficient $\Phi_{\text{norm}}$.\textsuperscript{17,19} Formally,

$$
\Phi_{\text{norm}}(k) = \frac{\Phi(k)}{\Phi_{\text{random}}(k)}
$$

A normalized coefficient $\Phi_{\text{norm}}$ greater than 1 over a range of $k$ suggests the existence of rich club organization in a network\textsuperscript{17,18} To this end, for each network, a population of $m=1,000$ random networks\textsuperscript{20} were computed by shuffling the links in $M$, preserving the weights of the connections as well as the (binary) degree sequence and thus all node degrees (including the hubs) in the network.\textsuperscript{20} This algorithm, introduced by Maslov and Snappen\textsuperscript{21} does not preserve the weight distribution of the nodes. For each random network the rich club coefficient $\Phi^w_{\text{random}}$ was computed over all levels of $k$ and $\Phi^w_{\text{random}}(k)$ was computed as the average rich club coefficient over the 1,000 random networks. For convenience, in the main text $\Phi^w_{\text{norm}}(k)$ was referred to as $\Phi^w(k)$. Note that the normalized rich club coefficient is invariant for an overall connectivity of the network (see the description of equation 1), enabling the possibility of comparing $\Phi^w$ between groups.\textsuperscript{16}

Graph analysis of structural connectome topology

Graph organizational metrics. Characteristic graph metrics were computed to examine possible differences in overall connectome topology in patients compared to controls. Graph metrics used in this study are described in detail elsewhere.\textsuperscript{16,20} Examined metrics included the (node-specific) degree strength $S$ (computed as the sum of the weights of the node’s connections), the clustering
coefficient $C$ (reflecting the level of local connectedness of a node), the shortest path length $L$
(reflecting the average minimal travel distance between nodes in the network), the global efficiency
(reflecting the capacity for network-wide communication) and the modularity (reflecting the level
of community structure in the network, measured as Newman’s modularity metric\textsuperscript{22}). As is typically
done, $C$ and $L$ were normalized to the clustering coefficient $C_{\text{random}}$ and shortest path length
$L_{\text{random}}$ of a set of random networks (preserving the weights, network density and degree
sequence of the network, 1,000 random networks were formed), providing the normalized
clustering coefficient $\gamma$ and normalized path length $\lambda$, respectively. All graph metrics
and null models were computed using the MATLAB-based Brain Connectivity Toolbox.\textsuperscript{20}

Statistical comparison: permutation testing

To evaluate the statistical relevance of observed effects, permutation testing was used for
randomizing group assignment.\textsuperscript{1,23} Permutation testing yielded an empirical null distribution of
effects under the null hypothesis that patient and control group were not different.\textsuperscript{1,23} First, for each
permutation, two random groups were formed, randomly assigning each patient and control data set
to each of two random groups (keeping group sizes intact). Second, for each examined graph metric
(e.g. connectivity strength/density of rich club, feeder and local connections, clustering-coefficient,
shortest path length, etc.), the difference between the means of the individual values of the two
created random groups was computed. For statistical testing of the rich club curves $\Phi^w$ the area-
under-the-curve of the individual rich club curves for a range of increasing $k$ was evaluated.\textsuperscript{24} Third,
step 1 to 3 was repeated for a total of 10,000 permutations, obtaining a distribution of differences
under the null hypothesis, i.e. indicating effects that were not related to disease status. Fourth, for
each graph metric of interest, using the obtained null distribution, the original difference between
the patient and control group was assigned a (two-tailed) p-value by computing the percentage of
the null distribution that exceeded the empirically measured metric (multiplied by 2 to obtain a two-
tailed p-value).
eResults

Effects of motion, global mean correction and Fisher-r-to-z transformation on resting-state fMRI data

Considering recent concerns of motion-related artifacts introducing spurious effects into functional connectivity (in particular effects related to age related differences),25,26 a post-hoc analysis was performed to examine the impact of motion in our study. To this end, the “scrubbing” procedure as suggested by Power and colleagues was used, removing scan frames from the individual time-series in which significant movement was detected.26 This procedure resulted in removing on average around 20% of the scan frames of both healthy controls and patients, comparable to results reported by Power and colleagues. Using the “scrubbed” time series, an identical procedure to compute resting-state functional connectivity and functional networks was applied (described) below. “Scrubbing” the time series did not have an effect on the results reported in this paper, suggesting no significant influence of motion effects on our reported data.

In addition, considering suggestions that global signal regression of the resting-state time series may introduce artifactual correlations to the data,27,28 a second post hoc analysis was performed in which global mean correction was excluded from the analysis. As a result, functional correlations were no longer centered around a mean of zero. Analyzing functional data (including structural-functional coupling) without global mean correction did not change the nature of our findings.

Note that in the main analysis, no Fisher r-to-z transform to the functional connectivity values was performed. This as the global signal regression already resulted in normal distributed data points. As a result, applying a Fisher r-to-z transform to the functional connectivity data before computing the SC-FC coupling did not change the nature of our presented findings, for the principal nor for the results of the replication data set.
Effect of rescaling of the structural weights to SC - FC coupling

Rescaling of the structural weights to a Gaussian distribution\(^{15,29}\) was used to normalize the distribution of structural connectivity values. To test the influence of this preprocessing step on our reported increase in SC - FC coupling, an additional analysis was performed in which this step was left out. In this post-hoc analysis the SC - FC coupling was computed as the correlation between streamline densities (streamline count dividing off regional volume) and FC values. Similar to the main analysis, increased SC-FC coupling was found in patients (mean/std, controls : 0.19/0.0642, patients : 0.22/0.0613, \(p = 0.033\), 10,000 permutations, cortex network).

Connectivity density results at other group rich club levels

Rich club regions as described throughout the main report were selected on basis of the group-averaged cortical network, set at a rich club level of \(k > 15\), as reported before.\(^{16,30}\) To examine the potential influence of the \textit{a priori} selected rich club level of \(k > 15\), the effect of reduced connectivity density of rich club connections in patients was also examined for other rich club levels. For rich club levels \(k > 11\) to \(k > 22\), i.e. selecting rich club members as those nodes in the group consensus matrix that displayed a degree of \(> k\) connections, the individual level of connectivity density of rich club connections was computed across patients and across controls. This resulted in individual rich club connectivity density values for all patients and for all controls at each examined rich club level. Possible group differences, per rich club level, were examined through means of permutation testing (10,000 permutations). eFigure S1 shows the observed differences between patients and controls at each rich club level. eFigure 1 shows for group rich club levels \(k > 11\) to \(k > 22\) the average rich club connectivity density for the group of controls (dark green) and group of patients (light green), the group differences (blue line) and the statistical p-value (permutation testing, 10,000 permutations). eFigure 1 also includes information on the number of nodes included in the rich club at each level, showing, as expected, that lower rich club levels (e.g. \(k > 12\)) are related to a relative larger rich club, while higher rich club levels (e.g. \(k > 20\)) result in a relative small, and more
exclusive rich club. Results at $k>15$ (i.e. 8 rich club members) are the results presented and discussed in the main text. eFigure 1 shows that rich club differences were present at multiple rich club levels, showing significant differences in rich club connectivity density in the range of $k>14$ to $k>20$ (all $p<0.001$, uncorrected for multiple testing). Note that the rich clubs at levels 14 and 15, levels of 17, 18 and 19, and 21 and 22 include the same number of members, and therefore involve identical rich clubs.

**Density ratios of rich club, feeder and local connections**

Global connectivity (i.e. total $S$), as well as rich club connectivity (both in absolute streamlines as well as connectivity density) were found to be reduced in patients as compared to controls (see main text). To examine whether abnormal connectivity might, to some extent, be concentrated to rich club connections, an additional analysis was performed in which the ratios of densities between rich club and feeder connections and between rich club and local connections were tested. For each individual data set the average density for each connection class (i.e. rich club, feeder, local) was computed and the ratios ‘rich club/feeder’ and ‘rich club/local’ were computed. These individual ratios (providing a value for each individual data set, for both controls and patients) were then tested between the two groups using permutation testing, using a similar procedure as described in the main text (10,000 permutations). Confirming our findings of the main analysis, both rich club/feeder as well as rich club/local ratios were significantly decreased in patients ($p=0.036$ and $p=0.024$ respectively, 10,000 permutations, one-tailed $p$-value). These findings tend to suggest that, although connections are affected throughout the brain network, connectivity effects might be concentrated to rich club connections.

**Individual rich club selection: connectivity density analysis**

Rich club regions as described throughout the main report were selected on basis of the group-averaged cortical network, set at a fixed rich club level of $k>15$. This rich club definition resulted
in the selection of 8 rich club nodes, which were then used as an *a priori* rich club across the individual networks (both patients and controls). This *a priori* rich club selection was used to avoid a (potential) bias towards the notion of patients having less hubs and therefore (as a rather trivial result) less inter-hub connectivity. However, as an alternative approach to this *a priori* rich club selection, one could also select the rich club purely on an individual basis, i.e. selecting a rich club in each individual based on its own structural connectivity network and use this rich club definition to select the densities of rich club, feeder and local connections (which may then vary according to the nodes included in the individual rich club). The influence of this alternative rich club selection procedure on our presented group results was examined in the following post-hoc analysis. Two approaches were examined: (1) selection of the rich club as the top 8 highest ranking nodes (i.e. 12%) of the network and (2) selection of the rich club as those nodes that display 1.25 standard deviations above the mean degree $k$ of the network. In contrast to (1) the latter procedure allows for a varying rich club size.

(1) *Individual rich club selection on basis of the top 8 highest ranking nodes.* For each individual data set, an individual rich club was selected as the subset of 8 top ranking nodes (i.e. top 12% nodes) with the highest degree in the individual structural network. Next, using this individual rich club, edges of the network were classified into rich club, feeder and local connections, and the density (i.e. number of streamlines dividing off regional volume) within each connection class was computed. This procedure was repeated for each individual data set (both patients and controls). Group differences in individual rich club, feeder and local densities were examined through means of permutation testing. *Results:* eFigure 2a shows the results of this analysis. Rich club density was significantly lower in patients as compared to controls ($p < 0.001$, 10,000 permutations), while less pronounced (and non-significant) differences were detected in feeder ($p = 0.110$) and local ($p = 0.778$) connections.

(2) *Individual rich club selection on basis of high degree nodes.* For each individual data set separately, an individual rich club was selected as those nodes with a degree $k$ of 1.25 standard
deviations above the mean degree of the network. Rich club size across the patient and control population is shown in eFigure 2b, with most patients and controls showing a rich club of 8 members. Rich club size did not differ between patients and controls (median controls: 8, patients: 8, p=0.850, t-test). Next, similar to above (analysis 1) density of rich club, feeder and local connections were computed and examined between groups. eFigure 2b displays the results, showing that rich club density was significantly lower in patients than in controls (p = 0.034, 10,000 permutations). No significant differences were detected in feeder (p = 0.801) and local (p= 0.240) connections.

Taken together, the results of analyses (1) and (2) are consistent with the results of the main analysis (i.e. selecting the rich club a priori on basis of the group-averaged network), suggesting that the selection procedure of the rich club did not have a strong influence on the observed affected rich club connectivity in patients with schizophrenia.

Rich club connectedness

For the connectivity density analysis, examining the connectivity density of rich club, feeder and local connections between patients and controls, it was examined whether the rich club comprised a single connected component across the group of subjects. With the 8 rich club members as described in the main text, in 80% (36 of 45) of the controls and in 77% (37 of 48) of the patients the rich club formed one single connected component. In the other 20% of the controls and 23% of the patients, the rich club consisted of one large connected component of 7 nodes and a small single node component: in 4 healthy control subjects this single-node component comprised the the left insula, in 5 subjects it involved the right insula; in 4 patients the single-node component involved the left insula, in 7 participants the right insula.

SC-FC coupling for rich club, feeder and local connections
Correlating the weights of the total class of connections with their level of functional connectivity revealed an increased level of SC-FC coupling in the patient population. To examine whether, and if so to what extent, this increase in SC-FC coupling might be related to a specific class or group of connections or whether it reflects a more global effect the following analysis was performed. First, for each individual network, the structural rich club, feeder and local connections were selected. Second, for each of these three connection classes separately, the weights of the edges were correlated with their functional counterparts from the functional connectivity matrix. Third, this was repeated for all individual data sets, obtaining a SC-FC coupling metric for each of the three classes for each subject. Potential differences in SC-FC coupling for each of these three classes between patients and controls was assessed using permutation testing (10,000 permutations per class).

**Results.** The SC-FC coupling was found to be increased for local connections (cortical networks, mean/std, patients: 0.295/0.0644, controls: 0.243/0.069 $p = 0.010$, 10,000 permutations). No significant differences (either increased or decreased SC-FC coupling) were found on feeder (cortical networks, mean/std, patients: 0.207/0.104, controls: 0.2191/0.087, $p = 0.61$, 10,000 permutations) or rich club connections (cortical networks, mean/std, patients: 0.237/0.284, controls: 0.303/0.257 $p = 0.288$, 10,000 permutations). Interestingly, if anything, the SC-FC coupling of rich club and feeder connections tend to show an opposite effect as compared to the local connections, being higher in controls than in patients, but care should be taken as these effects were clearly not significant. One has to take into account the difference in the number of connections included in each of the three classes, and therefore the number of observations on which the SC-FC correlation is computed. This is likely to explain the higher variation of the SC-FC coupling values in feeder and particular rich club connections, potentially limiting the findings of increased or decreased SC-FC coupling in these classes of connections. Future studies are needed to examine a potential different disease effect on SC-FC coupling between rich club, feeder and local connections.

*Rich club connections of frontal members*
Previous network studies have reported affected connectivity of both frontal as well as parietal regions in schizophrenia.\textsuperscript{1,23,31-33} However, effects have been suggested to be most pronounced for frontal hubs.\textsuperscript{1,23,32} To examine whether the rich club findings would be mostly driven by frontal members of the rich club, the following exploratory analysis were performed. (1). For all rich club connections (i.e. those white matter pathways that span between rich club members) separately, a simple t-test of streamline density was performed between patients and controls. No specific role of frontal connections could be reported; all effects were distributed over the entire rich club. (2) One at the time, each connection in the rich club (for both patients and controls) was disregarded from the analysis and the group analysis of comparison of rich club density between the group of patients and controls was re-run. This to examine whether one specific rich club connection had a disproportional effect on the reported rich club effects (i.e. when this connection would be removed from the analysis, the difference between patients and controls should become less pronounced). All runs (i.e. within each run removing a different rich club connection) revealed a significant decrease (p < 0.05, 10,000 permutations) in rich club density in patients, suggesting no disproportional effect to one specific rich club pathway. These findings tend to suggest that all rich club connections contribute to the overall effect, supporting our hypothesis of a reduced density of the rich club communication backbone as a whole in schizophrenia.

\textit{Influence of regional volume on rich club formation}

In this study a parcellation of the cortex into 68 (82 regions in the whole brain analysis) regions was used, based on the Freesurfer parcellation scheme. The Freesurfer parcellation is based on the gyrification pattern of the human cortex, dividing the cortex into distinct anatomical regions based on the boundaries between sulci and gyri.\textsuperscript{9} As a result, regions are not uniform in volume, with some regions involving a higher volume than others. Combined with the DTI based streamline tractography one could argue that this might introduce a (potential) bias towards finding a rich club formation of the human cortex, as larger regions might have a higher probability of obtaining a
higher degree, as more fiber streamlines are likely to start or terminate in these regions. To control for this potential bias towards more connectivity between regions with a high volume, streamline count between each pair of regions was corrected by dividing off their combined regional volume, as previously performed by others (e.g.,\textsuperscript{15,16}), obtaining a measure of streamline density. However, it has been noted that this might not completely remove the potential bias, as this correction cannot be applied to the connection count for region pairs where no connections were observed. A few remarks can be made. First, studies have suggested an association between the cortical folding pattern of the human cortex and their white matter connections.\textsuperscript{34,35} In this context, one might argue that the volume of certain gyri/sulci might be related to their number of white matter pathways to other brain regions, thereby introducing a potential causal relationship between large-scale brain hubs and white matter connectivity. Second, studies have shown rich club formation in the macaque and cat cortex,\textsuperscript{36,37} studies based on tract tracing data, not on \textit{in vivo} diffusion data. Third, human in vivo DTI studies have also shown rich club formation at a more fine-grained parcellation of the human cortex, minimizing the influence of regional volume on rich club formation.\textsuperscript{30}

To provide more insight into the (potential) influence of regional volume on rich club formation, the following analysis were performed. Analyses were performed on a group average connectivity matrix of the group of controls. Volume of a region was computed as the average volume of that particular region over the group of control subjects. Performing the following analysis on the group average matrix of the patients showed similar results.

\textit{Analysis I}. First, nodes were ordered according to their regional volume and associated to their binary nodal degree (i.e. the number of edges in the network of each node) as well as their sum of streamline density. Second, the streamline density of each \textit{connection} was plotted against the sum of volume of the two regions it connected to examine whether high density connections were indeed more likely to be formed between regions with high volume. \textit{Results}: eFigure 3 shows both plots. As expected, binary nodal degree was indeed associated to regional size, but effects were rather marginal (\(p = 0.003\)). Second, being the main topic of our study, the nodal sum of streamline density...
density was not found to be related to regional volume, showing that the applied correction indeed correct for differences in volume: larger brain regions did no longer have a higher level of overall density. Furthermore, eFigure 3c shows the relationship between regional volume and connectivity density split out for each edge in the network. No clear relationship between connectivity density and volume of the target and source node could be found, indicating that high volume regions are not, by definition, directly linked by denser connections, suggesting a minimal influence of regional volume on rich club formation on the group level.

**Analysis II.** As mentioned, volume correction cannot correct for region pairs in which no (binary) connection was found. However, although regional volume might have a (potential) influence on nodal-degree (and therewith rich club participation) this does not necessarily mean that regions with the highest volume show by definition (significant) rich club formation. To examine this, the following analysis was performed. First, regions were ranked to their total volume. Second, the top 8 nodes with the highest regional volume were selected. Third, streamline density and Φ between these 8 nodes was computed using equation 1 (main text). Fourth, a set of 1,000 random networks was computed (randomizing the connections of the group matrix, preserving the individual weights and overall degree sequence and\(^\text{20}\)) and the streamline density and Φ between these 8 largest nodes was computed, providing a null-distribution of connectivity density that could occur between the top ranking regions with the highest cortical volume. **Results:** The set of top 8 nodes with the highest volume included the left and right superior frontal cortex (also included in the rich club), left precentral gyrus, left inferior parietal cortex, bilateral rostral middle frontal gyrus and left and right superior parietal cortex (also included in the rich club). The Φ/Φ\(_{\text{random}}\) of these top 8 nodes was found to be higher than 1 (1.52), but with Φ only different from the null distribution of Φ\(_{\text{random}}\) on trend level (p = 0.08, 1,000 random networks for Φ\(_{\text{random}}\)). This in contrast to the Φ/Φ\(_{\text{random}}\) of the top 8 highest degree ranking nodes (i.e. the ‘true’ rich club), who showed a Φ/Φ\(_{\text{random}}\) of 1.78 with Φ being statically different from the null distribution of Φ\(_{\text{random}}\) (p = 0.002). Furthermore, as the top 8 highest volume regions also contained 4 of the rich club regions (i.e. superior frontal and superior...
parietal cortex, which might explain the still relative high $\Phi$ of the top 8 highest volume regions) a second analysis was performed in which $\Phi$ of the top 8 largest non-rich club nodes (i.e. bilateral superior frontal cortex, bilateral precentral gyrus, bilateral inferior parietal gyrus, bilateral rostral middle frontal gyrus) was computed. In this case, $\Phi/\Phi_{\text{random}}$ was found to be 1.31 with $\Phi$ not significantly different from $\Phi_{\text{random}}$ ($p = 0.36$). These analyses suggest that, although regional volume is related to a higher binary degree of nodes, the largest regions in the brain do not by definition show significant rich club formation.

Taken together, the findings of these two analyses tend to converge to the notion that rich club formation is not fully explained by effects in volume. Such a conclusion is strengthened by the reported rich club formation at higher parcellation resolutions\(^{16,30}\) together with reports of rich club formation of the mammalian cortex based on tract tracing data\(^{36-38}\) and rich club formation of the neural systems of the C Elegans worm,\(^{39}\) connectome data that is not directly influenced by the size of the selected network nodes.

Influence of region location on rich club formation

Besides volume, also the spatial location of regions in the brain can have an influence on rich club formation, as longer tracts might be more difficult to trace. Indeed, rich club regions have been hypothesized to be distributed over the entire cortex, with rich club connections spanning mostly long distances.\(^{30,36}\) Confirming previous findings, also in this data set, examining the physical length of the traced streamlines, rich club connections were found to be significantly longer than feeder and local connections (mean/std: rich club: 96.7/44.2, feeder: 59.6/38.9, local 43.7/32.9 mm | rich club vs feeder = $p<0.001$, rich club vs local = $p < 0.001$). We refer to\(^{30,36}\) for a detailed examination and discussion of the spatial embedding of the rich club in the human and macaque cortex.
Rich club results in schizophrenia sample (excluding patients with diagnosis of schizoaffective disorder)

The (principal) data set of 48 patients included 13 patients with the diagnosis of schizoaffective disorder and 1 patient with schizophreniform disorder. To test whether the observed rich club findings were also present in the subset of n=34 patients with the diagnosis of schizophrenia, all analyses were re-run with the group of n=34 schizophrenia patients. In summary, all findings, including rich club organization, rich club, feeder and local densities and SC-FC coupling were also found to be present in this sub-sample. These analyses included, in more detail, the following results:

Rich club organization. Similar to the results of the complete data set (n=48), schizophrenia patients (n=34) showed significantly reduced rich club organization as compared to controls (cortical: p = 0.001 for range k=15 to k=32, whole brain: p = 0.01 for range k=28 to k=29, 10,000 permutations). Dividing off effects of regional volume did not change the nature of these findings.

Rich club, feeder and local density. Density of rich club connections was found to be significantly reduced in the sub-sample of patients (p=0.0024, 10,000 permutations), while no such an effect was found to be present for feeder (p=0.200) or local (p=0.367) connections. Also the ratios of densities of rich club/feeder (p = 0.0624 ns) and rich club/local (p = 0.0170) were found to be lower in patients as compared to controls.

SC-FC coupling. Similar to the results of the complete data set, the subset of patients showed a higher level of SC-FC coupling (mean/std, patients:0.28/0.057, controls:0.25/0.048, p = 0.0061, 10,000 permutations) as compared to the group of healthy controls. Also the SC-FC coupling in the whole brain network was found to be increased in the subset of 34 patients (mean/std, patients:0.21/0.052, controls:0.19/0.049, p = 0.036, 10,000 permutations).

eReferences


Figure 1. Rich club connectivity density effects at different rich club levels. Rich club regions as described throughout the main report were selected on basis of the group-averaged cortical network, set at a rich club level of k>15. eFigure 1 shows the influence on the reported rich club connectivity density effects for different group-based rich club levels. eFigure 1 shows for group rich club levels k=11 to k=22 the average rich club connectivity density for the group of controls (dark green), group of patients (light green), the group differences (blue line), the statistical p-value of this difference (permutation testing, 10,000 permutations) and the number of rich club members, per rich club level (varying from 24 to 2). Significant reduced rich club density in patients was found at a range of levels, from k=14 to k=19 (all p < 0.01, 10,000 permutations), indicating that rich club differences are also present at other group rich club levels.
Figure 2. Connectivity density of rich club, feeder and local connections with the rich club defined on an individual basis. In the main analysis, the brain's rich club was a priori defined on basis of the group-averaged network at a rich club level of \( k = 15 \). However, as an alternative approach, differences in connectivity density between patients and controls was also examined with the rich club selected on an individual basis, selecting the rich club within each individual dataset separately as (1) the top 12\% highest (degree based) ranking nodes (panel A) and/or (2) nodes with a degree higher than 1.25 standard deviations of the average degree of the network (panel B). Consistent with the findings reported in the main text (i.e. rich club selection on a group basis), both individual approaches showed significant reduced rich club density in patients as compared to controls (A: \( p = 0.0018, \) B: \( p = 0.0390 \) indicated by *, 10,000 permutations), while the effect was less pronounced or not present in the feeder and local connections (A: feeder \( p = 0.113, \) B: feeder \( p = 0.049 \) | A: local, \( p = 0.732, \) B: local \( p = 0.245, \) 10,000 permutations).
Figure 3. Relationship between regional volume and connectivity. (A) Figure 3a shows the relationship between nodal degree (binary) and regional volume, confirming a relationship between volume of a node and its binary degree. (B) Figure 3b shows the relationship between nodal strength (sum of streamlines) and regional volume, showing a clear relationship between nodal volume and streamline density. (C) Figure 3c shows the relationship between nodal strength (streamline density) and regional volume, showing that dividing off regional volume corrects for the influence of regional volume on connectivity strength. Figure 3d shows, over all edges in the network, the association between connectivity strength (streamline count) and sum of the volume of the target and source nodes, showing no clear relationship. Figure 3e shows the same plot as figure 3d, but now with streamline density. Panels D and E illustrate that two high volume regions do not show a high streamline connectivity and/or high connectivity density per se.