The autism- and schizophrenia-associated protein CYFIP1 regulates bilateral brain connectivity and behaviour

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Copy-number variants of the CYFIP1 gene in humans have been linked to autism spectrum disorders (ASD) and schizophrenia (SCZ), two neuropsychiatric disorders characterized by defects in brain connectivity. Here, we show that CYFIP1 plays an important role in brain functional connectivity and callosal functions. We find that Cyfip1-heterozygous mice have reduced functional connectivity and defects in white matter architecture, similar to phenotypes found in patients with ASD, SCZ and other neuropsychiatric disorders. Cyfip1-deficient mice also present decreased myelination in the callosal axons, altered presynaptic function, and impaired bilateral connectivity. Finally, Cyfip1 deficiency leads to abnormalities in motor coordination, sensorimotor gating and sensory perception, which are also known neuropsychiatric disorder-related symptoms. These results show that Cyfip1 haploinsufficiency compromises brain connectivity and function, which might explain its genetic association to neuropsychiatric disorders.
The corpus callosum (CC) is the largest axonal commissure in the human brain and connects both cerebral hemispheres. Appropriate callosal microstructure and function are required for bilateral functional connectivity\textsuperscript{1,2}. Agenesis of the corpus callosum (ACC), callosotomy or defects in callosal integrity have been linked to deficits in social communication\textsuperscript{3}, social behaviour\textsuperscript{4}, motor coordination and learning\textsuperscript{5,6}, and cognitive performance\textsuperscript{2}. Similarly, CC abnormalities have been found in patients with diverse neuropsychiatric disorders such as autism spectrum disorders (ASD), schizophrenia (SCZ), fragile X syndrome (FXS), attention-deficit hyperactivity disorder (ADHD), Prader–Willi syndrome and 22q11.2 deletion syndrome\textsuperscript{7–13}. Interestingly, over the last decade, magnetic resonance imaging (MRI) studies in patients with ASD and SCZ have identified functional connectivity and microstructural defects in the white matter as a hallmark of these disorders. Previous work based on resting-state functional MRI (rsfMRI) revealed that brain functional connectivity (FC) is altered in ASD and SCZ patients, showing predominantly reduced long-range functional connectivity\textsuperscript{12–15}. Additionally, recent studies using diffusion-tensor imaging (DTI) showed that axonal integrity and connectivity are also affected in patients with ASD and SCZ\textsuperscript{16–18}. In particular, DTI revealed abnormalities in the corpus callosum of these patients\textsuperscript{16,19}. Furthermore, reduced myelination has also been observed in patients with ASD and SCZ\textsuperscript{20,21}.

Recently, copy-number variations (CNVs) of the chromosomal region 15q11.2 have been associated with the development of neuropsychiatric disorders, especially ASD and SCZ, and domain-specific cognitive impairments\textsuperscript{22}. The proximal region of the long arm of chromosome 15 contains several breakpoints, resulting in different rearrangements\textsuperscript{23}. The smallest region that has been linked to neuropsychiatric disorders is contained between the breakpoints BP1 and BP2, i.e., the 15q11.2 locus. CNVs of this region are quite frequent (~1% of the population\textsuperscript{24}) and cause a small but significant increase in the risk of developing ASD or SCZ\textsuperscript{25,26}. In all, 15q11.2 deletion and duplication carriers show abnormally volume of the corpus callosum among other cerebral and white matter abnormalities\textsuperscript{27–30}. The chromosomal region contained in the BP1–BP2 encodes four genes: TUBGCP5, NIPA1, NIPA2 and CYFIP1. Rare CNVs, single-nucleotide polymorphisms (SNPs) and point mutations single out CYFIP1 as the gene of that region most likely associated with SCZ\textsuperscript{31–33} and ASD\textsuperscript{34,35}. CYFIP1, the Cytoplasmic FMRP interacting protein 1, first described as “specifically Rac1-associated” (Sra-1) protein\textsuperscript{36}, is part of the Wave Regulatory Complex (WRC), a heteropentamer formed by WAVE1/2/3, CYFIP1, ABI1/2, NCKAP1 and HPSC300\textsuperscript{37,38}. The WRC functions as an Arp2/3 regulator, promoting its actin-nucleating activity, and therefore controlling actin assembly\textsuperscript{37}. This process can be activated by Rac1 signalling, which promotes a conformational change in CYFIP1\textsuperscript{39} and its binding to the WRC\textsuperscript{38}. In its alternative conformation, CYFIP1 binds FMRF and the cap-binding protein eIF4E, repressing protein translation of specific mRNAs\textsuperscript{40}. Both processes, actin dynamics and regulated protein synthesis, are crucial for synaptic development and brain functioning, strengthening the hypothesis that alterations in the CYFIP1 gene can account for the clinical features of patients with ASD and SCZ.

Deletion of the Cyfip1 gene in mice leads to embryonic lethality\textsuperscript{38,41,42} while heterozygous mice show aberrant behaviour\textsuperscript{41,43,44}, decreased dendritic complexity and increased number of immature spines\textsuperscript{38,42,45}. Cyfip1 heterozygous mice also present electrophysiological defects, such as enhanced mGluR-dependent LTD\textsuperscript{41} and vesicle release probability\textsuperscript{46}. These observations suggest that Cyfip1 heterozygous mice are a suitable model for the neurological defects observed in BP1–BP2 haploinsufficient patients. Recently, it was shown in zebrafish that CYFIP1 regulates axonal growth of retinal ganglion cells (RGSs)\textsuperscript{47} and Cyfip1 overexpression in mice leads to an increased proportion of mature spines\textsuperscript{48}. The role of Cyfip1 in brain connectivity, however, remains unexplored. Here, we show that Cyfip1 heterozygous mice (Cyfip1\textsuperscript{+/−}) have reduced functional connectivity and white-matter architecture defects. Cyfip1 haploinsufficiency leads to a decreased transmission of callosal synapses, reduced myelination in the corpus callosum, and behaviourally manifests with deficits in motor coordination as well as other traits reminiscent of ASD and SCZ-like symptoms. Together, our findings suggest that CYFIP1 is involved in the abnormal brain structures and callosal defects detected by MRI in individuals with the 15q11.2 deletion, and possibly in other forms of neuropsychiatric disorders with reduced CYFIP1 levels.

**Results**

**Bilateral functional connectivity is impaired in Cyfip1\textsuperscript{+/−} mice.** Many neuropsychiatric disorders are characterized by impaired brain connectivity. To investigate whether and how Cyfip1 haploinsufficiency could affect brain networks in vivo, we analysed functional connectivity (FC) in several brain regions using resting-state functional magnetic resonance imaging (rsfMRI). Cyfip1\textsuperscript{+/−} adult mice showed decreased FC compared to wild-type (WT) (compare the lower left and upper right halves of the matrix, Fig. 1a). Brain areas that presented significant difference between WT and Cyfip1\textsuperscript{+/−} mice are the cingulate cortex (Cg) and the thalamus (T) (Fig. 1b and Supplementary Fig. 1a). FC networks are characterised by midline symmetry, and correlations between homotopic regions are particularly strong\textsuperscript{49}. To analyse bilateral FC, seed-based analysis was performed across brain areas (Fig. 1c). Remarkably, FC with the contralateral side of the seed region was particularly affected in the hippocampus, somatosensory and motor cortices (Fig. 1d). In conclusion, our results show significant defects in functional connectivity, especially of the bilateral connections.

**Aberrant white matter architecture in Cyfip1\textsuperscript{+/−} mice.** Bilateral connectivity mostly passes through the corpus callosum and abnormalities in this brain structure are regarded a hallmark of neuropsychiatric disorders. To determine whether the observed functional deficits are due to structural axonal abnormalities in the CC, we performed diffusion tensor imaging (DTI)\textsuperscript{50} in WT and Cyfip1\textsuperscript{+/−} animals. DTI is one of the most widely used techniques to analyse white matter architecture and integrity. Among the parameters obtained with DTI, fractional anisotropy (FA) is the most widely used. High FA values are indicative of fibre-like structures and therefore, FA provides an estimate of axonal architecture\textsuperscript{51}. In Fig. 2a, the FA values across WT and Cyfip1\textsuperscript{+/−} brains were colour-coded, with higher values in red highlighting white matter tracts such as the CC. The differential map of WT and Cyfip1\textsuperscript{+/−} FA values showed that the highest reduction in FA was detected in the CC, in particular in the genu, its anterior part (Fig. 2b; first image). In addition, the statistical map showed that several brain regions have a nominally significant difference in FA between WT and Cyfip1\textsuperscript{+/−} brains (Fig. 2c). Again, these results pointed to the corpus callosum as the most affected white matter tract. We therefore calculated the DTI parameters in the CC (Supplementary Fig. 1b) and no statistical differences were observed in the mean (MD), axial (AD), or radial diffusivity (RD). In contrast, fractional anisotropy was significantly lower in the CC of Cyfip1\textsuperscript{+/−} mice (Fig. 2d), which might indicate changes in axonal thickness or myelination. Of note, we found a trend of increase (t-test with Holm-Sidak’s correction, p = 0.195) in radial diffusivity, suggesting defects in myelination\textsuperscript{50}. In support of this, we observed that the volume of
the corpus callosum was reduced in Cyfip1+/− mice compared to WT (Supplementary Fig. 1c).

To determine which axonal property (between axonal thickness and myelination) was affected, we performed electron microscopy of the corpus callosum. Representative micrographs from the anterior part of the CC were selected (Fig. 3a), and myelinated axons were automatically identified and parameterised. For each axon, the diameter and the myelin thickness were determined. In addition, the g-ratio, i.e., the ratio of the internal over the external axon diameter (Fig. 3b) was calculated.

We observed no changes in the axonal diameter between the two genotypes, whereas the myelin thickness was reduced (Fig. 3c). Consequently, the g-ratio increased across all axon sizes (Fig. 3c).

In conclusion, Cyfip1 haploinsufficiency causes defects in functional connectivity, especially of the callosal projections that connect the two cortical hemispheres, which correlates with a reduced myelin thickness of the callosal fibres.

**Cyfip1+/− mice have altered callosal presynaptic function.** Changes in myelin thickness might be due to abnormal number of oligodendrocytes, which are the cells responsible for axonal myelination51. However, the number of mature (myelinating) and immature oligodendrocytes in the corpus callosum revealed no
difference between WT and Cyfip1+/− mice (Supplementary Fig. 2), suggesting that this is not likely to be the primary cause of the observed reduction in myelin thickness.

It is well-established that neuronal activity can regulate callosal myelination. To investigate whether Cyfip1 deficiency leads to defects in neuronal activity, we measured spontaneous activity in the somatosensory cortex of acute brain slices using microelectrode arrays (MEAs) (Fig. 4a). We found that both the spike and burst rate were significantly reduced in the Cyfip1+/− cortices (Fig. 4b-d), indicating that there are perceptible network alterations in the adult brain. This reduction in spontaneous activity could explain the reduced myelin thickness of callosal axons, which would in turn affect their function, potentially having repercussion on synaptic neurotransmission. To study the latter, we extracellularly stimulated the corpus callosum to elicit EPSCs in pyramidal cells of the layer II/III, where contralateral projections arrive through the callosal tract (Fig. 5a). Upon challenging callosal axons with trains of stimulation, slices from WT mice presented short-term synaptic depression. In contrast, in Cyfip1+/− slices we observed significantly reduced frequency depression together with an increased EPSC2/EPSC1 ratio (Fig. 5b, c), indicating altered presynaptic function. Altogether, this evidence corroborates the hypothesis that in Cyfip1+/− mice, bilateral transmission via callosal tracts is impaired.

![Fig. 2](https://example.com/image2)

**Fig. 2** Cyfip1+/− mice show defects in callosal architecture. a Diffusion Tensor Imaging (DTI) of WT and Cyfip1+/− mice at postnatal day 60 (P60). Fractional anisotropy (FA) maps in which average FA values for WT and Cyfip1+/− mice are represented by a colour scale (WT n = 7 and Cyfip1+/− n = 6 mice). b Upper, FA differential maps between WT and Cyfip1+/− mice (Δ(WT−Cyfip1+/−)). Lower, representative anatomical MRI images as reference. c Statistical map of the FA differences between WT and Cyfip1+/− mice. Shown are the T-values in a colour scale ranging from 2.2 to 3.3 (equivalent to uncorrected p values ranging from 0.05 to 0.005). The red scale indicates reduced FA values in the Cyfip1+/− mice, the blue scale increased values. d The graph shows mean diffusivity (MD), axial diffusivity (AD), radial diffusivity (RD) and fractional anisotropy (FA) in the corpus callosum (WT n = 7 and Cyfip1+/− n = 6 mice) (mean ± SEM; two-tailed t-test; FA * p = 0.0165)
Cyfip1+/− mice show motor coordination defects. Callosal abnormalities have been associated with motor deficits, particularly with motor coordination, both in humans and in mice. We therefore used the accelerating rotarod and the ladder rung walking tests to investigate how the impaired bilateral connectivity and callosal transmission in Cyfip1+/− mice affect motor function. As shown in Fig. 6a, b, Cyfip1+/− mice fell off significantly earlier from the rotarod than their WT littermates, indicating reduced motor coordination. Of note, this defect was more prominent in the first trial (Fig. 6b). Interestingly, in the ladder rung walking test, Cyfip1+/− mice performed strikingly worse, slipping more often across trials (Fig. 6c), and in each of the three trials (Fig. 6d), than their WT littermates. To exclude that the abnormal coordination was due to reduced locomotion or muscle strength, general activity and strength were measured in the open field and hanging wire, respectively. Cyfip1+/− animals moved normally (Supplementary Fig. 3a–c) and had normal muscle strength (Supplementary Fig. 3d). Altogether, these findings indicate that the defects on the rotarod and the ladder rung test are indeed due to impaired motor coordination.

Cyfip1+/− mice recapitulate ASD and SCZ-like behaviours. To investigate whether this model has translational relevance, we analysed whether the Cyfip1+/− mice would recapitulate some ASD and SCZ-like behavioural phenotypes. Individuals with ASD are characterized by defects in social communication and repetitive and stereotyped behaviours. In addition, they present a wide range of symptoms such as defects in motor coordination, altered sensory processing, novelty seeking and decreased cognitive flexibility. We measured sensory perception/novelty detection using the texture novel object recognition paradigm (tNORT). In contrast to WT, Cyfip1+/− mice showed no preference for the novel vs. the familiar texture (Fig. 7a), suggesting defects in sensory processing or novelty seeking behaviour. Repetitive behaviour was assessed through scoring grooming behaviour and marble burying. In both tests Cyfip1+/− mice scored similarly to WT mice (Supplementary Fig. 5).}

Individuals with schizophrenia present symptoms that are generally grouped in three categories: positive symptoms (e.g., hallucinations), negative symptoms (e.g., affective blunting) and cognitive impairment (e.g., working memory deficits). In addition, sensorimotor gating defects are one of the most robust behavioural phenotypes found in schizophrenic patients. Sensorimotor gating is measured by prepulse inhibition (PPI), i.e., the attenuation of a startle response when preceded by a weaker prestimulation. PPI is regarded as an endophenotype of SCZ and can be easily translated to preclinical models to describe SCZ-like behaviour in rodents. We found that Cyfip1+/− mice have reduced PPI, similarly to what has been observed in patients with SCZ, and indicating decreased sensory motor gating (Fig. 7b, c).
Finally, we analysed working memory in the Y-maze, where Cyfip1\textsuperscript{+/−} mice showed no deficits compared to WT (Supplementary Fig. 4c, d).

In sum, we gathered compelling electrophysiological, anatomical, functional and behavioural evidence, which all demonstrate defects in callosal connections of the adult Cyfip1\textsuperscript{+/−} mouse brain. Furthermore, the Cyfip1\textsuperscript{+/−} haploinsufficiency mouse model recapitulates several behavioural endophenotypes of ASD and SCZ.

Discussion

Here, we describe an impairment in corpus callosum architecture and transmission in a mouse model of Cyfip1 haploinsufficiency. These callosal defects have an impact on functional connectivity and behaviour that might explain the genetic association of CYFIP1 with neuropsychiatric disorders.

We observed impaired bilateral functional connectivity (Fig. 1), which correlates with alterations in the callosal axons. Specifically we observed defects in the microstructure of the corpus callosum, determined by DTI, which correlates with reduced myelin thickness (Figs. 2 and 3), and presynaptic transmission (Fig. 5). Regarding presynaptic function, we observed that trains of stimulation lead to short-term synaptic depression in WT but not in Cyfip1\textsuperscript{+/−} slices (Fig. 5). This could be caused by changes in neurotransmitter release probability\textsuperscript{68}, which would be reduced in Cyfip1\textsuperscript{+/−} slices, although we cannot exclude that other presynaptic alterations could also be the cause. Arguably, changes in synaptic transmission have an impact on spontaneous activity, as we observed (Fig. 4), and neuronal activity modulates myelination of the callosal axons\textsuperscript{52}. In this model, the primary defect lies in the callosal axons. Alternatively, alterations in oligodendrocytes may represent the primary cause of the reduced myelination, which in turn would affect propagation of electrical signals along the callosal axons. We did not observe defects in oligodendrocyte number, suggesting that presynaptic dysfunction is likely to be fundamental in the callosal dysfunction. Interestingly, a recent in vivo study in zebrafish shows that reduced vesicle release leads to impaired myelination by reducing oligodendrocytes myelinating capacity\textsuperscript{69}. However, further studies are needed to elucidate this cause-consequence relation and the molecular role of CYFIP1 in presynaptic function.

Even though the largest reduction in FA in the Cyfip1\textsuperscript{+/−} mice was observed in the CC, other axonal tracts may also be affected. CYFIP1 is expressed across the whole brain\textsuperscript{42,70} and therefore axonal defects in the Cyfip1\textsuperscript{+/−} mice may be generalised. Indeed, both fractional anisotropy and functional connectivity seemed generally reduced across the whole brain in Cyfip1\textsuperscript{+/−} mice. Similarly, defects in FC and white matter architecture have been reported across the brain in patients with ASD and SCZ, as well as 15q11.2 deletion and duplication\textsuperscript{12,15,17,18,30}, suggesting that
connectivity problems between brain areas may also contribute, in addition to the callosal defects, to neuropsychiatric disorder-related behaviours. Importantly, patients with the 15q11.2 BP1–BP2 microdeletion syndrome present thinner corpus callosum. Notably, motor coordination deficits have been observed in patients with ASD40, and a high proportion of 15q11.2 patients (42%) show motor delay24, which may correlate with the reduced motor coordination we observed (Fig. 6). Autism spectrum disorders are a complex group of disorders characterized by social deficits, language impairment and repetitive behaviours. However, patients with ASD also present abnormal sensory processing58, novelty seeking61, working memory71, cognitive flexibility59 and sensorimotor gating72. Some of these phenotypes have also been associated with schizophrenia64,65.

Cyfip1 haploinsufficiency recapitulates several ASD and SCZ-related behavioural alterations, specifically in sensory perception/novelty seeking and sensorimotor gating (Fig. 7) as well as defective cognition and sociability41,43. These observations suggest that the Cyfip1+/− mouse model has face validity for ASD and SCZ.

Several other behavioural functions appear normal, such as working memory, cognitive flexibility and repetitive behaviours (this study and41,43) as well as general locomotion (this study and41,43, but see also44). While this work was under revision73, a study was published showing motor defects in the Cyfip1+/− mice48. Of interest, deficits in motor coordination, sensory perception and sensorimotor gating have been observed in other mouse models for ASD and SCZ. For instance, reduced sensorimotor gating and motor coordination have been observed in the SCZ mouse model for 22q11.2 deletion74,75. In addition, disruption of the ASD-associated Shank3 gene in mice leads to reduced texture discrimination and motor coordination62,76.

Of note, the BP1–BP2 region contains four genes; here, we show that in rodents the callosal defects and behavioural alterations are caused by the sole deletion of the Cyfip1 gene. While we cannot formally exclude a role for the other three genes in the region, our findings here, as well as human genetic evidence (see Introduction), indicate that human CYFIP1 could be a causative gene in the BP1–BP2 region.

Alterations in the callosal region are not only found in patients with 15q11.2 BP1–BP2 deletions and duplications27–30, they are also a hallmark of ASD19 and SCZ16. Thus, variations in other chromosomal regions genetically associated with ASD or SCZ present similar defects in brain structure and connectivity as the Cyfip1 haploinsufficient mice. For instance, 22q11.2 deletion carriers, a chromosomal rearrangement that is associated with SCZ, present structural abnormalities in several brain regions77 which are recapitulated in a mouse model for the disease (Df(16)A+−/−)78. Similarly, studies in humans carrying the autism-associated 16p11.2 microdeletion showed reduced prefrontal connectivity and microstructural defects in the corpus callosum. These defects were also found in the mouse model for the disease, in which the callosal abnormalities were associated with increased axonal g-ratio79. Furthermore, Cntnap2 KO mice, one of the first identified genes associated with ASD80, have defects in callosal transmission and cortical myelination53, as well as reduced functional connectivity and aberrant g-ratio of the callosal axons81,82. Taken together, these studies point out that neuropsychiatric disorders of different genetic origins present similar defects in brain connectivity and white matter architecture, especially in the corpus callosum, highlighting the importance of brain connectivity as a possible point of convergence for several neuropsychiatric disorders83. In addition, some of the areas that showed reduced functional connectivity in the Cyfip1+/− mice are part of the default mode network (DMN), a brain network that reflects functional connectivity across different brain regions during conscious-inactive tasks84. Although the exact function of the DMN is still unknown, DMN defects have been found in patients with SCZ and ASD and have been suggested to be key for understanding the pathophysiology of these disorders15,85.

**Fig. 5** Reduced presynaptic function in Cyfip1+/− adult mice. a Schematic of the experimental setup. b Representative sample traces induced in layer II/III cortical neurons at P60 by trains of callosal stimulation (5 pulses, 20 Hz). Recordings obtained from WT (black) and Cyfip1+/− (grey) mice. c Left, graph of the normalised amplitude of EPSCs, and right, histogram and scatter plot of the amplitudes of the fifth EPSC, relative to the first. (WT n = 8 cells, three mice and Cyfip1+/− n = 12 cells, three mice; mean ± SEM; two-way repeated measures ANOVA, genotype main effect **p = 0.0017, F(1,18) = 13.53, and interaction p < 0.0001; two-tailed t-test **p = 0.0046)
In conclusion, our results suggest that CYFIP1 is most likely the gene in the 15q11.2 region which haploinsufficiency is responsible for key phenotypes of patients with ASD and SCZ. CYFIP1 haploinsufficiency leads to alterations in anatomic and functional connectivity, thereby explaining the increased incidence of these pathologies in patients with 15q11.2 deletions. Importantly, this pathological mechanism might not be limited to neuropsychiatric disorders caused by CNVs in the 15q11.2 region. CYFIP1 protein has been found dysregulated in SCZ independently of mutations in the CYFIP1 locus, indicating that other genetic and/or environmental factors may converge on the regulation of CYFIP1 abundance or activity, rendering the protein a hub for the development of SCZ and perhaps also related disorders. Altogether, the connectivity deficits caused by CYFIP1...
deficiency could therefore have a major contribution in the pathogenesis of neuropsychiatric disorders.

Methods

Animal care. Animal housing and care were conducted according to the Belgian, Swiss and European laws, guidelines and policies for animal experimentation, housing and care (Belgian Royal Decree of 29 May 2013, European Directive 2010/ 63/EU on the protection of animals used for scientific purposes of 20 October 2010 and Swiss law fédérale sur la protection des animaux 455). All the experimental procedures performed in Switzerland complied with the Swiss National Institu- tional Guidelines on Animal Experimentation and were approved by the Cantonal Veterinary Office Committee for Animal Experimentation. In all cases special attention was given to the implementation of the 3Rs, 5% goal housing and environmental conditions and analgesia to improve the animals’ welfare.

A 12-h light/dark cycle was used, and food and water were available ad libitum. The Cyp2f1+/− mouse line, generated by gene trap at the Sanger Institute, UK, was kindly provided by Seth G.N. Grant. The gene trap cassette was inserted between exon 12 and 13. Molecular characterisation of the Cyp2f1+/− mice was previously reported by De Rubens et al.38, C57BL/6 wild type (WT) and Cypf1 heterozygous (Cypf1+/−) male mice of 2–4 months were used. To generate the experimental animals, Cypf1+/− male were crossed with WT C57BL/6 females. Animal manipulation was performed according to the ECD guidelines approved by the Institutional Ethical Committee of the KU Leuven and Cantonal Veterinary Office Committee for Animal Experimentation.

rsfMRI and DTI. Animals: for the MRI handling procedures, adult male mice were anaesthetised with 2.5% isoflurane (IsoFlo, Abbott, Illinois, USA), which was administered in a mixture of 70% nitrogen (400 cc min−1) and 30% oxygen (200 cc min−1). Resting state functional magnetic resonance imaging (rsfMRI) procedures were performed as previously described39. In brief, a combination of medetomidine (Domitor, Zoetis, Karlsruhe, Germany) and atipamezole (Antiseadan, Pfizer, Karlsruhe, Germany) was used to sedate the animals. After positioning of the animal in the scanner, medetomidine was administered subcutaneously as a bolus injection (0.3 mg kg−1), after which the isoflurane level was immediately decreased to 1%. Five minutes before the rsfMRI acquisition, isoflurane was decreased to 0.4%. rsfMRI scans were consistently acquired 40 min after the bolus injection, during which the isoflurane level was maintained at 0.4%. After the imaging procedures, the effects of medetomidine were counteracted by subcutaneously injecting 0.1 mg kg−1 atipamezole (Antisedan, Pfizer, Karlsruhe, Germany).

For the diffusion tensor imaging (DTI) measurements, after the handling procedures under isoflurane (2.5%), isoflurane levels were decreased to 1.5% and maintained throughout the scanning procedure. The physiological status of all animals was monitored throughout the imaging procedure. A pressure sensitive pad (MR-compatible Small Animal Monitoring and Gating system, SA Instruments, Inc.) was used to monitor breathing rate and a rectal thermistor with feedback controlled warm air circuitry (MR-compatible Small Animal Heating System, SA Instruments, Inc.) was used to maintain body temperature at (37.0 ± 0.5°C).

Imaging procedures: rsfMRI procedures were performed on a 9.4T Biospec MRI system (Bruker BioSpin, Germany) with the Paravision 5.1 software (www.bruker.com). Images were acquired using a standard Bruker cross coil setup with a quadrature volume coil and a quadrature surface coil for mice. Three orthogonal multi-slice Turbo RARE T2-weighted images were acquired to render slice-positioning uniform (repetition time 2000 ms, effective echo time 33 ms, 16 slices of 0.5 mm). Field maps were acquired for each animal to assess field homogeneity, followed by local shimming, which corrects for the measured inhomogeneity in a rectangular VOI within the brain. Resting-state signals were measured using a T2*-weighted single shot EPI sequence (repetition time and the matrix size (96 × 96)

Imaging procedures: rsfMRI and DTI data, including realignment, normalisation and smoothing (for the rsfMRI data), was performed using SPM8 software (Statistical Parametric Mapping, http://www.fil.ion.ucl.ac.uk). First, all images within each session were realigned to the first image. This was done using a least-squares approach and a 6-parameter (rigid body) spatial transformation. The obtained parameters of the rsfMRI data, motion parameters resulting from the realignment were included as covariates to correct for possible movement that occurred during the scanning procedure. Second, all datasets were normalised to a study-specific EPI template. The normalisation steps consisted of a global 12-parameter affine transformation followed by the estimation of the nonlinear deformation fields. Finally, in plane stretching was done for the rsfMRI data using a Gaussian kernel with full width at half maximum of twice the voxel size (0.31 × 0.62 mm²). All rsfMRI data were filtered between 0.01 and 0.1 Hz using the REST toolbox (RESTL7, http://resting-fmri.sourceforge.net).

Regions-of-interest (ROIs) were manually defined using the Franklin and Paxinos anatomical mouse brain atlas and the MRicron software (MRicron version 6.6, 2013, http://www.mccauslandcenter.sc.edu/mricorco): cingulate cortex, caudate putamen, hippocampus, motor cortex, orbitofrontal cortex, prelimbic cortex, retrosplenial cortex, somatosensory cortex and thalamus. The rsfMRI data were first analysed using a region-of-interest (ROI) correlation analysis, where pairwise correlation coefficients were calculated and z-transformed using an in-house programme developed in MATLAB (MATLAB R2013a, The MathWorks Inc. Natick, MA, USA). Mean z-transformed FC matrices were calculated for each group. For each brain region mean FC, i.e., mean FC of each region across the other regions in the matrix, and bilateral FC were calculated. Statistical analyses of the pairwise correlations included in the two-way ANOVA (with genotype and sex as factors) and the Holm-Sidak correction for multiple comparisons.

Next, a seed-based analysis was performed to identify functional correlations of each region to all the voxels in the brain. Seed-based analyses were performed by first computing individual z-transformed FC-maps of the respective region using the REST toolbox, after which mean statistical FC-maps were calculated for each group in SPMS.

Each individual DTI FA-map was warped to a reference anatomical image. Then, average FA-maps for WT and Cypf1−/− mice were computed and a differential map (∆(WT-Cypf1−/−)) was calculated. To assess statistically significant group differences between the two groups, we performed a voxel-wise two-sample t-test.

Finally, the corpus callosum was manually delineated and DTI parameters (i.e., radial diffusivity, axial diffusivity, mean diffusivity and fractional anisotropy) were computed with MATLAB and statistical analyses were performed using two- sample t-tests to compare group differences.

The volume of the corpus callosum was calculated using Amira5.4 software overlapping individual CC ROIs over each subjects T2-weighted anatomical MRI images.

Electron microscopy. Adult mice from both genotypes were perfused, via the heart, with 100 ml of a buffered mix of 2.5% glutaraldehyde and 2.0% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4), and then left for 2 h before the brain was removed, embedded in 5% agar in 1% agarose and sagittal vibratome sections cut at 80 μm thickness through the midline. These sections were then post-fixed in potassium ferrocyanide (1.5%) and osmium (2%), and stained with thiocticohydroxydrazine (1%) followed by osmium tetroxide (2%). They were later stained overnight in uranyl acetate (1%) and washed in distilled water at 50°C before being stained with lead aspartate at the same temperature. They were finally dehydrated in increasing concentrations of alcohol and then embedded in spurs resin and hardened at 65°C for 24 h between glass slides. The regions containing the corpus callosum were trimmed from the rest of the section using a razor blade and glued to a blank resin block. One-micrometre-thick sections were then cut from the block face and mounted onto silicon wafers of 1 cm diameter.

The sections were imaged inside a scanning electron microscope (Zeiss Merlin, Zeiss NTS) at a voltage of 2 kV and image size pixel of 7 nm. Backscattered electrons were collected with a Gatan backscattered electron detector with a pixel dwell time of 1 μs. Multiple images of the corpus callosum were collected and tiled using the TrakEM2 plugin88 in the FIJI software.

A custom-made MATLAB code was used to identify and parameterise myelinated axons within the EM images. Inner (axon) and outer (axon + myelin) areas were segmented and their corresponding circular equivalent diameters calculated. The g ratio was calculated as the ratio between the internal and external diameter (dI/d) (Fig. 3b).

Immunohistochemistry and confocal microscopy. Adult mice from both genotypes were transcardially perfused with cold 4% PFA (pH 7.4). Coronal sections were obtained using a cryostat (Leica CM 3050 S; 20 μm thick). Free-floating sections from the anterior part of the CC (bregma 1.1 mm to 0.74 mm) were permeabilized and blocked at RT in phosphate-buffered saline (PBS, pH 7.4), 0.5% Triton X-100, 0.5% BSA in PBS, normal serum. Slices were incubated overnight at 4°C with the primary antibodies (rabbit anti-Olig2 1:10000, AB9610 Millipore; mouse anti-CC-1 1.200, Sigma OP80) and 2 h at RT with the secondary Alexa-conjugated antibodies (Life Technologies). DAPI was used for nuclei visualisation. Brain sections were mounted using Mowiol (Sigma).

Confocal images were acquired using a Leica SP8 microscope. stacks images were processed using Cell-2 (Leica). For the analysis, a threshold was selected based on the light level of the fluorescence (set at 0.3 of the signal) and a 2.5 pixel wide region was selected on the interface between the CA2 and the CA3 region. The threshold value was adjusted for each sample. The number of Olig2+ cells was manually counted using the Image J software.

Microelectrode array recordings. Cortical slices were prepared from adult male mice to study the effects of Cypf1 haploinsufficiency in spontaneous activity. Briefly, the mice were anaesthetised with isoflurane and the brains were quickly
removed and placed in bubbled ice-cold 95% O2/5% CO2-equilibrated solution containing (in mM): NaCl 125, KCl 2.5, NaH2PO4 1, MgCl2 1.2, CaCl2 0.6, Glucose 11, NaHCO3 26 (95% O2, 5% CO2). Cortical slices were made using a vibratome (Leica Biosystem). Slices were kept for 30 minutes at 33 °C and for 1 hour at RT in artificial cerebrospinal fluid (ACSF) containing (in mM): NaCl 125, KCl 2.5, NaH2PO4 1, MgCl2 1.2, CaCl2 1.0, Glucose 11, NaHCO3 26 (95% O2, 5% CO2).

A single slice was placed in a multi-electrode array (MEA) chamber (Multichannel systems, Reutlingen, Germany) positioning the electrode array on the somatosensory cortex. Each MEA is composed of 59 Ti electrodes organised in an 8 × 8 grid layout. The electrode distance is 200 μm, and the electrode diameter is 30 μm and insulated in the MEA chamber using a silver paint. Each electrode was continuously perfused with modified ACSF containing (in mM): MgCl2 0.75, CaCl2 1.4, (95% O2, 5% CO2). Neuronal activity, sampled at 10 kHz and filtered at 100 Hz, was recorded with a MEA2100-acquisition System (Multichannel systems, Reutlingen, Germany). The data were acquired with the MultiChannel Expenderer 2.8 and extracted for analysis using the MultiChannel Analyzer 2.6 (Multichannel systems, Reutlingen, Germany). For spike detection, the threshold was set at −5.5 s.d. of the noise amplitude. An event was considered a burst if it was composed of at least five consecutive spikes with an inter-spike interval of <90 ms. Data formatting, detection of bursts and events as well as the statistical analyses were performed with a custom-written script in R (software version 3.4.3).

Patch clamp recordings. Animals were decapitated and the brain was quickly sliced. Slicing was performed in bubbled ice-cold 95% O2/5% CO2-equilibrated solution containing (in mM): choline chloride 110; glucose 25; NaHCO3 25; MgCl2 7; ascorbic acid 11.6; sodium pyruvate 3.1; KCl 2.5; NaH2PO4 1.25; CaCl2 0.5. Coronal slices (250 μm) were stored at −22 °C in 95% O2/5% CO2-equilibrated artificial cerebrospinal fluid (ACSF) containing (in mM): NaCl 124, KCl 26.2; glucose 11; KCl 2.5; CaCl2 2.5; MgCl2 1.3; NaH2PO4 1. Recordings (flow rate of 2.5 ml min−1) were made under an Olympus BX51 microscope (Olympus) at 32 °C. Currents were amplified, filtered at 5 kHz and digitised at 20 kHz. Access resistance was monitored by a step of −4 mV (0.1 Hz). Experimenters were discarding the data if the access increased more than 20%.

The bipolar stimulating electrode was placed in the corpus callosum, and EPSCs were evoked in layer II/III pyramidal neurons while holding the cells at −60 mV. Five pulses at 20 Hz were delivered at 0.1 Hz to measure paired pulse ratios. The internal solution contained (in mM): Ca2+ 10; Mg2+ 2; Hepes 10; EGTA 10; sodium creatine-phosphate 5; Na2ATP 4; Na2GTP 0.4; pH 7.3

**Behavioural assays. Open field:** exploration was observed in an open field setup with a square surface area of 45 cm². Mice were placed in the open field for 10 min and movements were recorded with EthoVision XT 14 tracking software (Noldus, Wageningen, The Netherlands). A central zone was defined as a square surface area 15 cm equidistant from all 4 walls of the open field. Total distance travelled as well as centre entries were measured.

**Rotarod:** motor coordination was tested on the accelerating rotarod as previously described. Briefly, mice were first trained for 2 min on the rotarod at constant speed (4 rpm). They were subsequently tested on four 5-min trials interleaved with 10 min rest. During the test trials, mice were placed on a rotating rod that accelerated from 4 to 40 rpm in 5 min and the latency to fall off the rod was recorded.

**Self-grooming:** animals were placed individually in a transparent cylinder (7 cm diameter, 20 cm high), and self-grooming activity was monitored for 10 min with a vibratome (Leica Biosystem). Distance travelled was measured. On the third day, a texture-based novel object recognition test was introduced. During the first phase, the mouse could freely explore for 10 min two objects that were identical in texture and appearance (Familiar; two 50 ml Falcon tubes wrapped with gradient 80 sandpaper). After 5 min retention period, the mouse was re-introduced in the arena. During the second phase, one of the objects was replaced by a visually identical but texturally different object (Novel; 50 ml Falcon tube wrapped in gradient 100 sandpaper) and mouse explorative behaviour towards the two objects (at a distance of 5 cm) was recorded. Exploratory behaviour was defined as close proximity towards the object (nose oriented towards the object at a body centre distance of <5 cm). The location of the novel object was counterbalanced between animals. The novelty preference index was calculated as Time novel/(Time familiar + Time novel) × 100. The position of the novel arm was counterbalanced between animals and the apparatus was cleaned 5% ethanol water solution between each animal.

The spontaneous alternations in the Y-maze measures short-term spatial novelty detection and is considered a test for working memory. Mice were placed in the middle of the maze and were allowed to freely explore all three arms for 5 min. The number of arm entries, as well as the number of alternations was recorded. An arm entry was defined as when all four feet were off the floor of the arm. An alternation is considered when, in a moving triple of arm visits, all three arms have been visited (e.g. 1-2-3, 1-3-2, 2-1-3, etc.). The alternation score was calculated as 100 × (number of alternations/number of visit triplets).

**Texture novel object recognition task (iNORT):** an adaptation of the novel object recognition task was used as previously described. Briefly, during the first two days of the experiment, mice were placed for 10 min inside an open field arena with opaque walls (45 × 45 × 45 cm) to ensure habituation to the environment before the test. The bottom of the arena was covered with bedding material. Mouse activity was tracked with EthoVision XT 14 software (Noldus, Wageningen, The Netherlands). Distance travelled was measured. On the third day, a texture-based novel object recognition task was introduced. During the first phase, the mouse could freely explore for 10 min two objects that were identical in texture and appearance (Familiar; two 50 ml Falcon tubes wrapped with gradient 80 sandpaper). After 5 min retention period, the mouse was re-introduced in the arena. During the second phase, one of the objects was replaced by a visually identical but texturally different object (Novel; 50 ml Falcon tube wrapped in gradient 100 sandpaper) and mouse explorative behaviour towards the two objects (at a distance of 5 cm) was recorded. Exploratory behaviour was defined as close proximity towards the object (nose oriented towards the object at a body centre distance of <5 cm). The location of the novel object was counterbalanced between animals. The novelty preference index was calculated as Time novel/(Time familiar + Time novel) × 100.

**Hanging wire test:** the test is adapted from Aartsma-Rus et al. In brief, mice, held at the base of the tail, were guided to grasp the midpoint of a wire (length: 60 cm, height: 30 cm, thickness: 3 mm) with both forepaws and then released. The average fall latency of each mouse was recorded. The test was repeated three times with a 30-s intertrial interval and a cut-off of 5 min was used.

**Ladder running test:** the ladder running test was adapted from previously described. Briefly, the setup consisted of two transparent sidewalls and metal rungs (2.7 cm diameter). Mice were inserted in the sides to create a horizontal ladder with a regular distance of 1 cm between rungs (regular interval variant). The two sidewalls were separated by 3 cm, creating a narrow corridor of 32 cm long and 10 cm high for the mouse to traverse towards a home cage at the end of the corridor. The ladder was elevated from the table by 20 cm. Animals trained to cross the ladder within 2 min from the start of the corridor to the safe home cage. Mice were motivated to cross the ladder and did not require further reinforcement. First, mice were allowed to freely explore the apparatus and walk along the corridor for 2 min. Afterwards, all animals were tested for 3 trials with an intertrial interval of 15 min.

A 17-cm-long section of the corridor was recorded with a horizontally placed camera and videos were analysed afterwards. The number of foot slips from both fore and hind paws and the total number of steps taken to traverse the corridor were measured. The proportion of foot slips per animal trial is reported. Foot slips were defined as a completely missing or falling off a rung while walking across the corridor.

**Touchscreen:** visual discrimination and reversal learning were tested using instrumental conditioning procedure using the touchscreens (Campden Instruments LTD, Loughborough, Leics., UK) as previously described. In brief,
mice were trained to discriminate two visual images presented at the same time. Correct choices were rewarded with strawberry milkshake, while incorrect choices were punished with 5 s timeout (indicated by chamber lights switching on). After training the animals to select and touch a visual image during a rigorous shaping procedure, animals were presented 2 visual stimuli (pairwise discrimination of correct and incorrect stimulus) that they needed to recognise and select to obtain a reward. After reaching criterion (80% correct trials on three consecutive days), the rule was reversed and the formerly incorrect stimulus was rewarded (reversal phase). Each session was limited to maximally 30 correct trial or 60 min. Number of trials to criterion was assessed for initial discrimination and reversal learning. Responses were recorded using the Abet II software.

**Statistics.** Statistical analyses were performed with SPSS and MATLAB for the MRI and DTI experiments, and with GraphPad Prism or R for EM, electrophysiology, and behavioural experiments. The statistical tests used are listed in the respective Figure legends. In brief, unpaired two-tailed Student’s t-tests or non-parametric two-tailed Mann–Whitney U-tests were used for comparisons between two groups. Multiple-t-test with Holm-Sidak multiple comparison correction was used for the rsfMRI experiments. For cumulative frequency distribution, Kolmogorov-Smirnov test was used. For comparisons of more than two groups Two-way ANOVA or Two-way Repeated Measures ANOVA with Holm-Sidak’s multiple comparison test was used. For all analyses, P-values < 0.05 were considered significant and annotated as follows: *p < 0.05, **p < 0.01, ***p < 0.001. Results were presented as mean ± standard error of the mean (SEM).

**Reporting summary.** Further information on research design is available in the Nature Research Reporting Summary linked to this article.

**Data availability**

All relevant data are available from the authors upon request.

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**Author contributions**
Conceptualisation by N.D.I. and C.B.; N.D.I. participated in all the experiments described in this work. MRI imaging by D.S. and A.V.d.L., electrophysiology by A.V., M.T. and M.M. Custom-written R scripts for MEA and behavioural experiments by A.C.L., Custom-made MATLAB code for EM analysis and DTI maps by M.A.; Y.M., D.G., R.D., T.A., K.W.L. and A.B.S helped with data analysis and manuscript preparation. A.R.S. and Z.C.V. helped with the behavioural experiments.

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