

ORIGINAL ARTICLE

Genetic deletion of fibroblast growth factor 14 recapitulates phenotypic alterations underlying cognitive impairment associated with schizophrenia

TK Alshammari^{1,2,3,10}, MA Alshammari^{1,2,3,10}, MN Nenov², E Hoxha^{4,5}, M Cambiaghi⁵, A Marcinno⁴, TF James⁵, P Singh⁶, D Labate⁶, J Li^{7,8}, HY Meltzer⁷, B Sacchetti⁵, F Tempia^{2,4,5} and F Laezza^{2,8,9}

Cognitive processing is highly dependent on the functional integrity of gamma-amino-butyric acid (GABA) interneurons in the brain. These cells regulate excitability and synaptic plasticity of principal neurons balancing the excitatory/inhibitory tone of cortical networks. Reduced function of parvalbumin (PV) interneurons and disruption of GABAergic synapses in the cortical circuitry result in desynchronized network activity associated with cognitive impairment across many psychiatric disorders, including schizophrenia. However, the mechanisms underlying these complex phenotypes are still poorly understood. Here we show that in animal models, genetic deletion of fibroblast growth factor 14 (*Fgf14*), a regulator of neuronal excitability and synaptic transmission, leads to loss of PV interneurons in the CA1 hippocampal region, a critical area for cognitive function. Strikingly, this cellular phenotype associates with decreased expression of glutamic acid decarboxylase 67 (GAD67) and vesicular GABA transporter (VGAT) and also coincides with disrupted CA1 inhibitory circuitry, reduced *in vivo* gamma frequency oscillations and impaired working memory. Bioinformatics analysis of schizophrenia transcriptomics revealed functional co-clustering of *FGF14* and genes enriched within the GABAergic pathway along with correlatively decreased expression of *FGF14*, *PVALB*, *GAD67* and *VGAT* in the disease context. These results indicate that *Fgf14*^{-/-} mice recapitulate salient molecular, cellular, functional and behavioral features associated with human cognitive impairment, and *FGF14* loss of function might be associated with the biology of complex brain disorders such as schizophrenia.

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INTRODUCTION

Psychiatric diseases such as schizophrenia, depression and bipolar disorder are associated with cognitive deficits thought to arise from an imbalance between the excitatory and inhibitory (E/I) tone in cortical circuits.^{1,2} Fast-spiking GABAergic parvalbumin (PV) interneurons have a fundamental role in maintaining E/I balance, controlling excitability and shaping the synaptic plasticity of principal neurons.³ Even though disrupted function of other types of inhibitory neurons has been associated with the etiology of brain disorders, changes in PV neurons are one of the most consistent associations. In animal models, reduced function of PV neurons results in desynchronized network activity, decreased gamma frequency oscillations and cognitive deficits, phenotypes that mimic clinical presentation and post-mortem tissue studies of virtually all human brain disorders associated with disrupted cognition.^{1,4–10}

In schizophrenia and bipolar patients, the number of PV interneurons and the expression level of molecular components of GABAergic synapses, such as glutamic acid decarboxylase 67 (GAD67) and vesicular gamma-amino-butyric acid (GABA) transporter (VGAT), are found decreased in post-mortem brains leading

to the ‘GABA hypofunction hypothesis’ as a potential etiology.^{1,11–16} Yet, the molecular understanding of how such a detrimental loss of the GABAergic system might lead to corrupted cortical networks manifesting in disease, remains poorly explored.

Recent large-scale genome-wide association studies identified *FGF14* as locus of single-nucleotide polymorphisms and as such a potential disease-associated gene for schizophrenia, bipolar disease, depression, epilepsy and addictive behaviors,^{17–27} corroborating initial reports of missense mutations in *FGF14* as genetic links to the neurodegenerative disorder, spinocerebellar ataxia 27.^{28,29} These results highlight the possibility of an as yet undiscovered and pivotal role for the *FGF14* gene in psychiatric disorders.

Highly expressed in the central nervous system, FGF14 is an accessory protein of voltage-gated Na⁺ (Nav) channels at the axonal initial segment (AIS),^{30–36} a regulator of neuronal excitability,^{30,37–39} a presynaptic organizer of glutamatergic synapses,^{40,41} a scaffolding molecule for kinase signaling pathways^{33,34,42,43} and a regulator of synaptic plasticity.^{41,44,45} At the circuitry and behavioral level, genetic deletion of FGF14 in *Fgf14*^{-/-} mice results in presynaptic structural deficits of CA3–CA1

¹Pharmacology and Toxicology Graduate Program, University of Texas Medical Branch, Galveston, TX, USA; ²Department of Pharmacology and Toxicology, University of Texas Medical Branch, Galveston, TX, USA; ³King Saud University Graduate Studies Abroad Program, King Saud University, Riyadh, Saudi Arabia; ⁴Neuroscience Institute Cavalieri Ottolenghi, Turin, Italy; ⁵Department of Neuroscience, University of Torino, Turin, Italy; ⁶Department of Mathematics, University of Houston, Houston, TX, USA; ⁷Department of Psychiatry and Behavioral Sciences, Northwestern University Feinberg School of Medicine, Chicago, IL, USA; ⁸Mitchell Center for Neurodegenerative Diseases, The University of Texas Medical Branch, Galveston, TX, USA and ⁹Center for Addiction Research, The University of Texas Medical Branch, Galveston, TX, USA. Correspondence: Dr F Laezza, Department of Pharmacology and Toxicology, University of Texas Medical Branch, 301 University Boulevard, Galveston, TX 77555, USA. E-mail: felaezza@utmb.edu

¹⁰These authors contributed equally to this work.

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hippocampal synapses, decreased long-term potentiation, and cognitive deficits, aberrant responses to epileptic agents and decreased threshold for seizure induction.^{41,44,45} Furthermore, *Fgf14*^{-/-} mice exhibit abnormal locomotor activity explained by a reduced response to dopamine receptor D2 agonists in the basal ganglia.⁴⁵ Both phenotypes in the hippocampus and the basal ganglia could imply an impaired GABAergic tone supporting the emerging view of a tight correlation between the dopamine and the GABAergic systems.^{46,47} Yet, evidence of FGF14 control of GABAergic inhibitory transmission is lacking.

Using a combination of confocal microscopy, patch-clamp electrophysiology, *in vivo* local field potential recordings and behavioral studies, we discovered that genetic deletion of *Fgf14* in rodents leads to a reduced number of PV interneurons, decreased expression of GAD67 and VGAT, and reduced GABAergic transmission in CA1 pyramidal neurons associated with impaired gamma oscillations and working memory. Further, bioinformatics analysis of schizophrenia transcriptomics confirms FGF14 functional clustering with GABAergic synaptic signaling and identifies genetic covariance of *FGF14*, *PVALB*, *GAD67* and *VGAT* in the disease, supporting *Fgf14*^{-/-} mice as an attractive model to interrogate the biology of complex brain disorders associated with disrupted cognitive circuitry such as schizophrenia.

MATERIALS AND METHODS

Animals

The primary *Fgf14*^{-/-} colony was maintained at the animal facilities of the University of Texas Medical Branch and of the University of Torino following approved protocols. Genotypes were confirmed by in-house PCR analysis or by Charles River Laboratories International (Houston, TX, USA). Description of the animal husbandry is given in the Supplementary Material.

Immunofluorescence

Brain tissue derived from *Fgf14*^{-/-} and *Fgf14*^{+/+} 4- to 5-month-old male littermates was processed for immunofluorescence staining using either 4% paraformaldehyde or liquid nitrogen vapor fixation followed by permeabilization, blocking, and primary and Alexa-conjugated secondary antibody staining as previously described⁴⁸ and outlined in the Supplementary Material.

Data acquisition and image analysis

Multichannel confocal images were acquired using a Zeiss LSM-510 META (Carl Zeiss Microscopy, Jena, Germany) confocal microscope as previously described.⁴⁹ Detailed information on image quantification can be found in the Supplementary Material.

Western blotting

Tissue homogenate preparation and western blotting were carried out as previously described⁵⁰ and outlined in the Supplementary Material.

Whole-cell patch-clamp electrophysiology

Horizontal hippocampal slices were prepared from 4- to 5-month-old *Fgf14*^{-/-} and *Fgf14*^{+/+} male littermates, and whole-cell patch-clamp recordings were performed at room temperature from CA1 pyramidal neurons using standard parameters previously described^{51,52} and outlined in the Supplementary Material.

In vivo electroencephalogram

Extracellular field potentials were recorded in freely behaving mice using stainless steel electrodes stereotaxically implanted in the CA1 of the right hippocampus.⁵³ Spectrograms were calculated using the software NeuroExplorer (Nex Technologies, Madison, AL, USA).

Behavioral tests

Tests were performed in *Fgf14*^{-/-} and *Fgf14*^{+/+} 2- to 4-month-old males using the 4–8 version of the eight-arm radial maze.⁵⁴ A detailed description of behavioral experiments and analysis are part of the Supplementary Material.

Human studies

As a computational gene co-expression search engine, SEEK (<http://seek.princeton.edu/>) was applied to identify a gene network co-expressed with FGF14. Functional annotation and pathway enrichment was performed with a built-in Gene Ontology and KEGG terms. Enriched data sets were downloaded from NCBI Gene Expression Omnibus, and the differential gene expression was analyzed by R Limma packages described in the Supplementary Material. We also applied GeneMANIA and STRING to the analysis of pathway enrichment and protein–protein interaction for the co-expressed genes of FGF14 in hippocampus. The method and result are detailed in the Supplementary Material.

RESULTS

Anatomical abnormalities found in the CA1 hippocampal region of post-mortem brains from schizophrenia patients have been identified as an index of the disease severity and treatment responsiveness.⁵⁵ These studies along with the reported association between hippocampal PV neurons with cognitive function in the normal brain and in schizophrenia, prompted us to begin our investigations in the CA1 hippocampal region.^{15,16,46,47,55} Thus, we first asked whether FGF14 was expressed in PV interneurons in the CA1 region. Confocal imaging confirmed that FGF14 is expressed at the AIS of cells across all CA1 sublayers (Figure 1a), including PV-positive neurons (Figure 1b). Therefore, we posited whether genetic ablation of *Fgf14* might have physiological consequences for these inhibitory neurons.

To test this hypothesis, we first examined whether the overall number of PV interneurons in the CA1 area was affected by genetic loss of FGF14. Cell count quantification based on immunofluorescence staining revealed that the total number of PV-positive cells was significantly reduced in *Fgf14*^{-/-} mice compared with wild-type controls (Figures 1c–e; 74.21% ± 5.02, 100% ± 4.89, $P < 0.001$) with a most pronounced and significant reduction in the stratum oriens (SO; 71.42% ± 6.96, 100% ± 8.10, $P < 0.05$) and in stratum pyramidale (SP; 77.94% ± 4.96, 100% ± 7.06, $P < 0.05$; Figure 1f). Notably, the expression level of PV per cell was not significantly different across groups ($P = 0.78$, $n = 4$ littermate mice; Supplementary Figures 1F–I); the neuropil composed of PV-positive neurites in either SP or SO was also unaffected by *Fgf14* deletion ($P = 0.49$ and 0.28 ; Supplementary Figures 1A–E). Furthermore, the total number of cells in the CA1 SP, which primarily includes pyramidal neurons, was also unchanged ($P = 0.24$; Figures 1j–l). Thus, we concluded that deletion of *Fgf14* leads to a cell- and subfield-specific reduction in the number of PV neurons in the CA1 hippocampal region.

PV interneurons are the primary source of GABAergic synapses in the CA1 region. Reduction in GABA synthesis and its synaptic release machinery accompany loss of PV neurons in schizophrenia and other psychiatric disorders.^{4–6} Consistent with this, GAD67 mean fluorescence intensity in PV soma, the primary source of the enzyme pool, and in puncta across SP was significantly reduced in *Fgf14*^{-/-} mice compared with littermate control (89.85% ± 3.08, 100% ± 3.96, $P < 0.05$; and 91.86% ± 0.10, 100% ± 0.08, $P < 0.0001$, respectively; Supplementary Figures 2A–D and Figures 2a–c). The area per puncta and total puncta number, though, were unchanged ($P = 0.10$ and 0.88 , respectively; Figure 2c). An additional marker and key regulatory protein at presynaptic GABAergic inputs is VGAT, the vesicular transporter that loads presynaptic vesicles with GABA, another marker associated with brain disorders.^{56–58} Along with GAD67, the mean fluorescence intensity of VGAT in PV soma and its mean content per puncta in

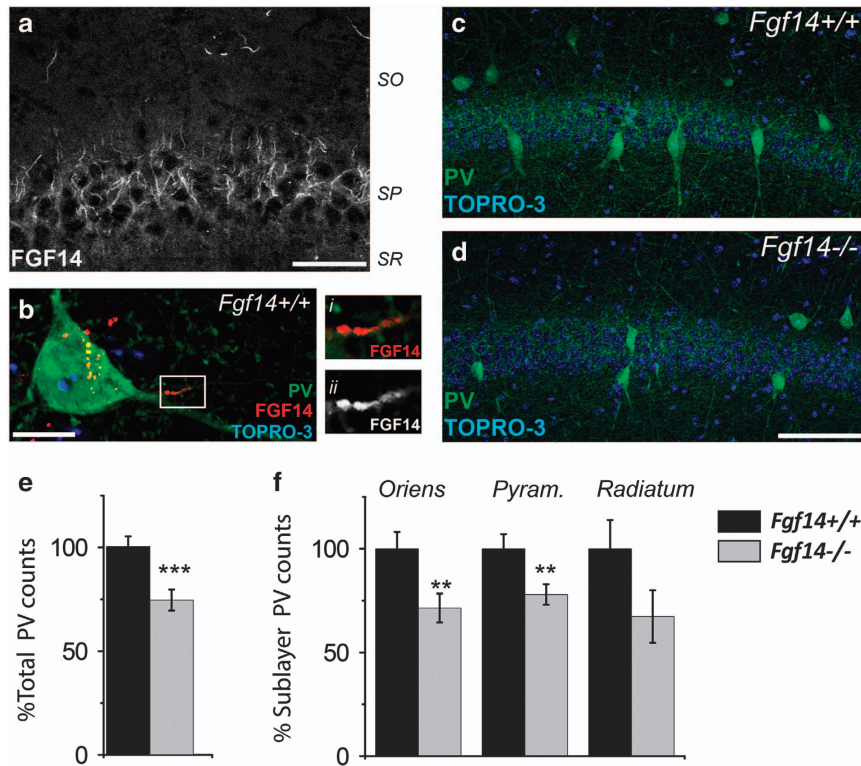


Figure 1. Genetic deletion of *Fgf14* results in structural changes in the CA1 parvalbumin (PV) interneurons. (a) FGF14 immunoreactivity is detectable at the axonal initial segment (AIS) of cells in CA1. (b) FGF14 (red) expressed in the soma and AIS of PV interneurons (green), i and ii represent zooms of the boxed area. (c, d) PV interneurons in the CA1 region of *Fgf14*^{+/+} and *Fgf14*^{-/-} mice and respective higher resolution views of PV somas (i and ii). (e, f) Quantification of total PV interneurons in CA1 (380 cells in *Fgf14*^{+/+} and 282 in *Fgf14*^{-/-}), and in specific subfields (oriens, pyramidalis and radiatum). Data represent mean ± s.e.m., ****P* < 0.001; ***P* < 0.02; **P* < 0.05 statistical differences were assessed by Student's *t*-test or non-parametric Mann–Whitney test. Scale bars, 40 μm (a); 10 μm (b); 100 μm (d).

SP were significantly decreased in *Fgf14*^{-/-} mice compared with littermate controls (88.12% ± 3.06 vs 100% ± 3.18, *P* < 0.01, *n* = 4 littermate mice and 91.92% ± 0.14 vs 100% ± 0.135, *P* < 0.001, *n* = 3 littermate mice, respectively; Supplementary Figures 2E–H and Figures 2d–f), while puncta area and number were unchanged (*P* = 0.66 and 0.79, *n* = 3 mice; Figure 2f). Consistent with these results, Western blot analyses of hippocampal cell lysate revealed a significant reduction of GAD67 and VGAT protein content in *Fgf14*^{-/-} animals compared with wild type (Figures 2g and h).

PV interneurons contribute greatly to the integrity of the GABAergic circuit in the CA1 region, posing the question of whether *Fgf14* deletion might result in functional consequences for inhibitory transmission. Using patch-clamp electrophysiology, we recorded spontaneous and miniature inhibitory postsynaptic currents (sIPSCs and mIPSCs, respectively) from visually identified CA1 pyramidal neurons in *Fgf14*^{-/-} and wild-type control mice (Figures 3a and f). We found that genetic deletion of *Fgf14* led to a rightward shift in the probability distribution of sIPSC frequency (apparent loss in the higher frequency domain) accompanied by a reduction in the largest- and smallest-size synaptic event population (Figures 3c–e; *P* < 0.001, Kolmogorov–Smirnov test). Spontaneous IPSCs are action potential-driven synaptic events and as such represent a compound readout of the firing status of presynaptic interneurons and their neurotransmitter release machinery. To examine GABA release mechanisms independently from interneuron spontaneous firing, we recorded mIPSCs in the presence of tetrodotoxin and found that, similarly to sIPSCs, the frequency distribution histograms showed lowered probability of short inter-event intervals in *Fgf14*^{-/-} when compared with wild-type control mice (Figures 3f–h, *P* < 0.001 with Kolmogorov–

Smirnov test). This phenotype was paralleled by a loss of large- and small-amplitude mIPSCs in *Fgf14*^{-/-} compared with wild-type control mice (Figures 3f, i and j). In both sIPSCs and mIPSCs, the averaged sIPSC frequency and amplitude between the two groups were not significantly different (Supplementary Figure 3) and no changes in rise and decay time were found across synaptic events (both sIPSCs and mIPSCs) of different genetic groups (Supplementary Figure 4). Thus, genetic deletion of *Fgf14* leads to functional changes in the CA1 inhibitory circuitry that support the structural alterations demonstrated in Figures 1 and 2. Moreover, it suggests loss of interneuron firing (shift in sIPSC frequency) and pre- and postsynaptic modifications at GABA synapses (shift in frequency and amplitude distribution of mIPSCs, respectively).

Reduced PV neuron function can desynchronize the CA1 network resulting in reduced gamma oscillations and impaired cognition. Thus, we postulated that gamma oscillations might be impaired upon ablation of *Fgf14*. To test this, gamma oscillations were recorded in the CA1 SR layer in *Fgf14*^{-/-} and control mice by *in vivo* local field potential (Figure 4a). Spectral analysis within the 30–100 Hz range revealed that *Fgf14*^{-/-} mice had a strong reduction in gamma oscillation power (2.94 ± 0.11 μV², *n* = 7 compared with 7.92 ± 0.17 μV², *n* = 7 in wild-type mice, *P* < 0.05; Figures 4b and c) with both slow (low gamma, 30–65 Hz) and fast (high gamma, 65–100 Hz) gamma oscillations significantly impaired in *Fgf14*^{-/-} mice compared with wild type (*Fgf14*^{-/-}, 2.29 ± 0.85 and 0.65 ± 0.25 μV²; wild-type control, 5.97 ± 1.2 and 1.95 ± 0.5 μV²; Figure 4d). Thus, consistent with disrupted GABAergic transmission, spectral analysis confirmed that FGF14 is required for the integrity of the cognitive circuitry. The

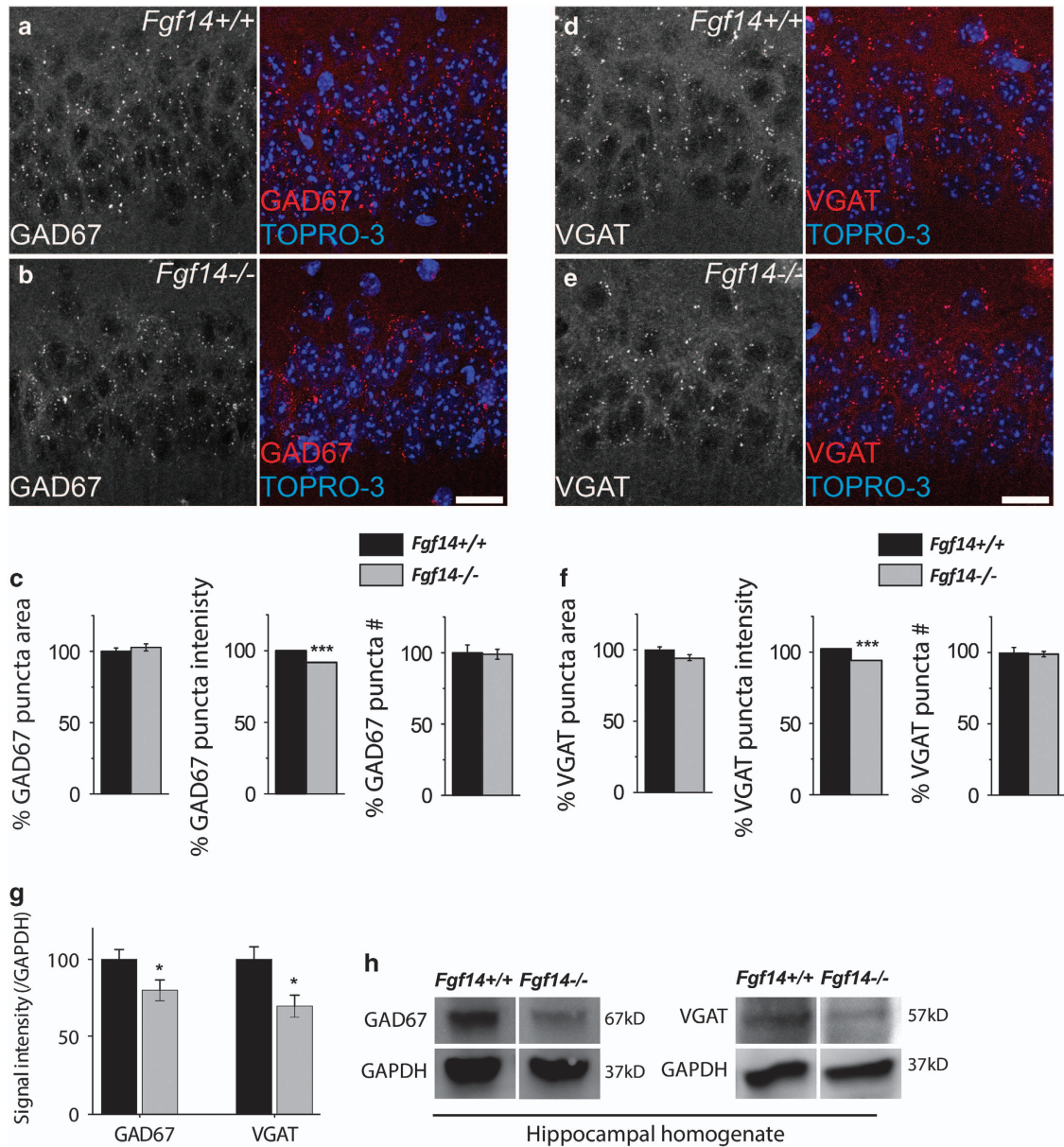


Figure 2. The effect of *Fgf14* genetic ablation on GABAergic presynaptic proteins in the hippocampal CA1 region. (a, b) GAD67 representative puncta in CA1 stratum pyramidale (SP) at high magnification. (c) Quantification of GAD67 puncta area, puncta intensity and puncta number in the indicated genotypes. (d, e) Vesicular GABA transporter (VGAT) representative puncta in CA1 SP at high magnification. (f) Quantification of VGAT puncta area, puncta intensity and puncta number in the indicated genotypes. (g) Quantitative western blot analysis of GAD67 and VGAT from the hippocampus. (h) Immunoblot detection of GAD67 and VGAT in whole hippocampal homogenates from *Fgf14*^{-/-} mice and *Fgf14*^{+/+} controls. Data represent mean \pm s.e.m., *** $P < 0.001$; ** $P < 0.02$; * $P < 0.05$ statistical differences were assessed by Student's *t*-test or non-parametric Mann-Whitney test. Scale bars, 20 μ m (a, d). GABA, gamma-aminobutyric acid; GAD67, glutamic acid decarboxylase 67.

combination of phenotypes observed in *Fgf14*^{-/-} mice (Figures 1–4) has been associated with deficits in spatial working memory in animal models and patients afflicted with schizophrenia.^{1,58,59} Thus, we next evaluated the spatial working memory performance of *Fgf14*^{-/-} mice using the eight-arm maze test. Analysis of the latency to perform the task showed that *Fgf14*^{-/-} mice required a longer time to complete the task than wild type ($n = 20$ wild type and $n = 19$ *Fgf14*^{-/-}, $P < 0.001$; Figure 4e). Furthermore, revisiting errors (Figure 4f), which are directly linked to working memory performance, were more frequent in *Fgf14*^{-/-} mice than in wild types ($P < 0.05$), corroborating the cellular and functional phenotypes associated with genetic deletion of *Fgf14*.

To provide translational value to our studies, we examined large transcriptomic data sets from schizophrenia post-mortem tissues deposited in the NCBI Gene Expression Omnibus, seeking genes whose expression might covary with that of *FGF14*. Through the SEEK-based gene co-expression search engine with built-in functional annotation and pathway enrichment by Gene Ontology terms (Supplementary Table 2) and by KEGG ortholog analysis, we found that *FGF14* was enriched within the 'GABAergic synapse' pathway (Supplementary Table 1) and its expression profile correlated with that of *PVALB* ($P = 0.004$ in hippocampus; $P = 0.0059$ in prefrontal cortex), *GAD67* ($P = 0.0009$ in hippocampus; $P = 0.0003$ in prefrontal cortex) and *VGAT* ($P = 0.04$ in

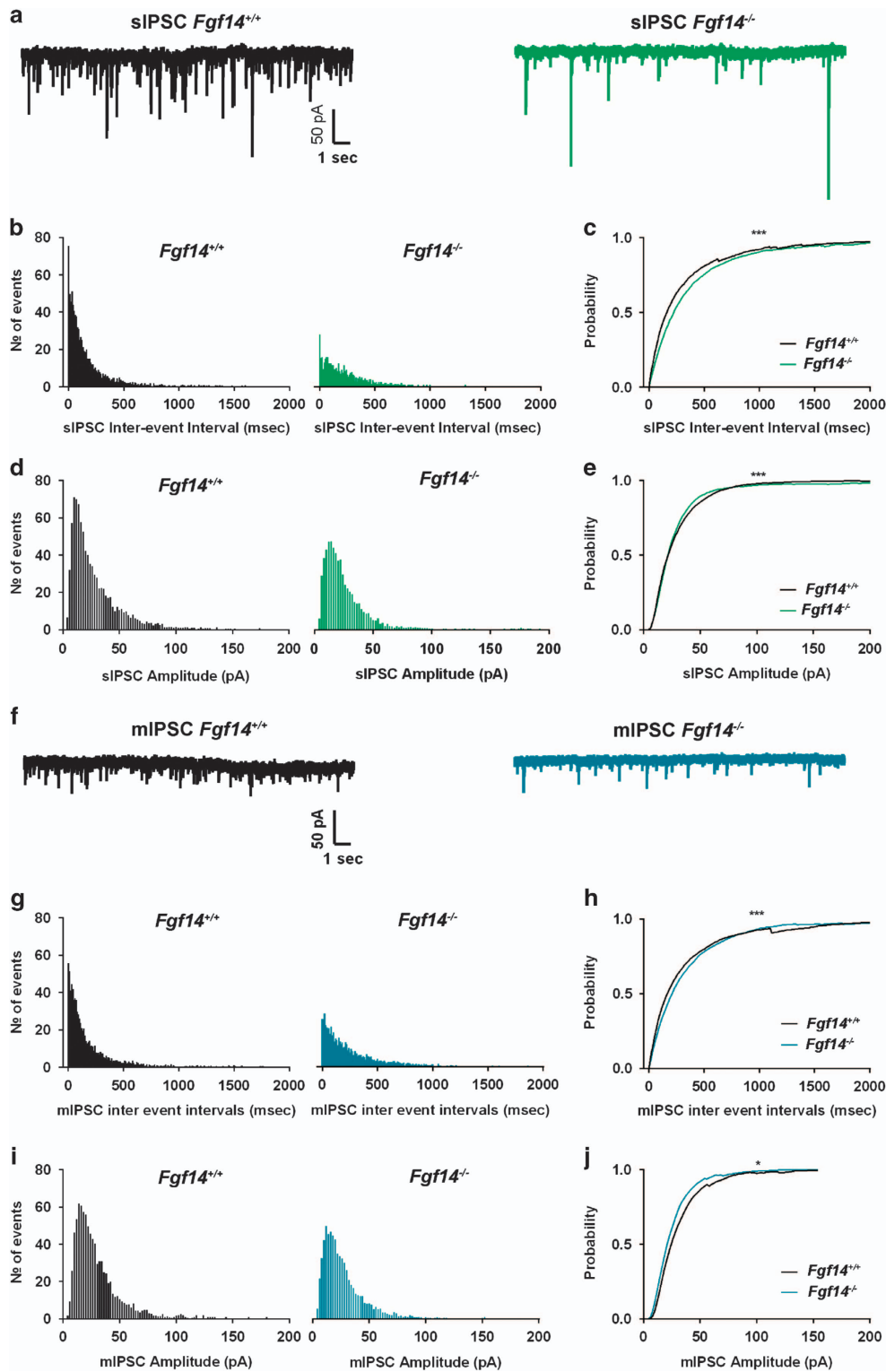


Figure 3. Genetic deletion of *Fgf14* impairs GABAergic transmission in the CA1 region. Representative traces of whole-cell patch-clamp recordings showing effect of *Fgf14* ablation on spontaneous inhibitory postsynaptic current (sIPSCs) (a) and miniature inhibitory postsynaptic current (mIPSCs) (f). (b) Inter-event-interval distribution of spontaneous GABAergic events in *Fgf14*^{+/+} (*n* = 8 cells) and *Fgf14*^{-/-} (*n* = 10 cells) mice. (c) Inter-event-interval cumulative distribution plot for *Fgf14*^{+/+} and *Fgf14*^{-/-} (sIPSCs; ****P* < 0.001, Kolmogorov–Smirnov test). (d) Amplitude distribution of spontaneous GABAergic events in *Fgf14*^{+/+} (*n* = 8 cells) and *Fgf14*^{-/-} (*n* = 10 cells) mice. (e) Amplitude cumulative distribution plot for *Fgf14*^{+/+} and *Fgf14*^{-/-} sIPSCs (****P* < 0.001; Kolmogorov–Smirnov test). (g) Inter-event-interval distribution of miniature GABAergic events in *Fgf14*^{+/+} (*n* = 6 cells) and *Fgf14*^{-/-} (*n* = 7 cells) mice. (h) Inter-event-interval cumulative distribution plot for *Fgf14*^{+/+} and *Fgf14*^{-/-} mIPSCs (****P* < 0.001 with Kolmogorov–Smirnov test). (i) Amplitude distribution of miniature GABAergic events in *Fgf14*^{+/+} (*n* = 6 cells) and *Fgf14*^{-/-} (*n* = 7 cells) mice. (j) Amplitude cumulative distribution plot for *Fgf14*^{+/+} and *Fgf14*^{-/-} sIPSCs (**P* < 0.05; Kolmogorov–Smirnov test).

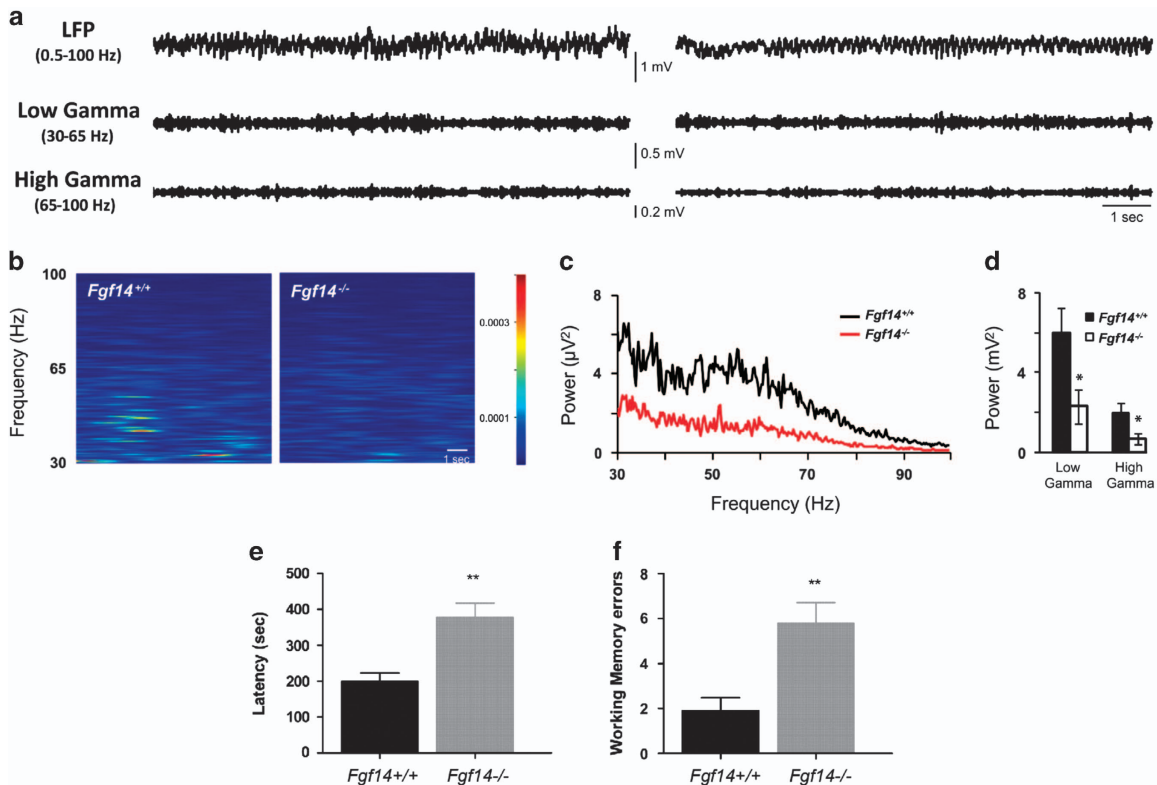


Figure 4. Genetic deletion of *Fgf14* reduces gamma frequency and affects working memory. (a) Representative traces of 10-s *in vivo* electroencephalogram recordings in the CA1 region of hippocampus (local field potential; LFP) in *Fgf14*^{+/+} (left) and *Fgf14*^{-/-} mice (right); filtered traces within low- and high-gamma band are also shown. (b) Spectrogram analysis of the above-mentioned traces in the gamma range (30–100 Hz). (c) Mean power spectral density of CA1 activity showing a marked decrease in gamma power in *Fgf14*^{-/-} ($n = 7$) with respect to *Fgf14*^{+/+} ($n = 7$) mice, as revealed by power analysis within both low- and high gamma (d). (e) *Fgf14*^{-/-} mice required a longer time to perform the eight-arm maze test ($n = 20$ wild type and $n = 19$ *Fgf14*^{-/-}; $P < 0.001$, *t*-test). (f) Analysis of working memory errors committed during the test day showed a significant difference between genotypes ($P < 0.05$, *t*-test). Data are expressed as mean \pm s.e.m. * $P < 0.05$.

hippocampus; $P = 0.069$ in prefrontal cortex) (Supplementary Table 3) in tissue/disease-specific conditions including schizophrenia. Western blot analysis confirmed a significant reduction of GAD67 and VGAT protein expression in the hippocampus (Figures 2g and h) as well as in the prefrontal cortex (Supplementary Fig. 5A, B) in *Fgf14*^{-/-} mice compared with controls, providing further correlations between our mouse model and human studies. We subsequently analyzed expression of the *FGF14*, *PVALB*, *GAD67* and *VGAT* genes and their correlations in two schizophrenia-enriched data sets and matched controls from the dorsal lateral prefrontal cortex.^{60,61} Both data sets showed a significant decreased expression of *FGF14*, *PVALB*, *GAD67* and *VGAT* (Figure 5a and Supplementary Figure 6), and a highly significant correlation between *FGF14*, *PVALB*, *GAD67* and *VGAT* was found in all samples and in schizophrenia alone with the largest effect size in schizophrenia and controls for *GAD67* (Figure 5b). The top enriched pathway such as 'GABAergic synapse' was also confirmed by another pathway enrichment tool, GeneMANIA and the GABAergic gene cluster was identified by STRING as showed in Supplementary Table 4 and Supplementary Figure 8.

DISCUSSION

Here we provide new evidence for FGF14 in maintaining GABAergic activity in the CA1 hippocampal region, an area critical for cognitive function.⁶⁵ Genetic deletion of *Fgf14* leads to a decrease in the number of PV interneurons and in the expression level of the presynaptic GABAergic markers GAD67 and VGAT.

These changes are associated with reduced inhibitory tone of pyramidal neurons, decreased gamma frequency oscillations and deficits in working memory. Bioinformatics analysis from human transcriptomics identified FGF14 as a component of GABAergic synaptic signaling and revealed a correlated decrease in *FGF14*, *PVALB*, *GAD67* and *VGAT* gene expression in schizophrenia post-mortem tissues compared with matched controls. These results provide a new mechanistic role for FGF14, an emerging neuropsychiatric disease-associated gene¹⁷⁻²⁷ in the context of human brain disorders.

The identification of FGF14 immunoreactivity at the AIS of CA1 PV interneurons suggests this protein contributes to interneuron structural and functional diversity⁶⁶ and as such might be part of the repertoire of signaling molecules dictating the cell cardinal and definitive specifications of PV neurons in the neural circuitry.^{1,67} The cell type and sublayer-specific loss of PV neurons observed upon *Fgf14* deletion confirms this hypothesis and supports the notion that FGF14 is indispensable for the development, maintenance and/or survival of PV interneurons in the CA1 region. Our post-mortem human tissue study identifies a significant correlation between the *Fgf14* and *PVALB* genes, which might contribute to the loss of PV interneurons observed in *Fgf14*^{-/-} mice.

We also found that the expression level of GAD67 and VGAT, two well-characterized disease-associated proteins critical for GABA synthesis and differentiation in addition to synaptogenesis of PV neurons,⁶⁸⁻⁷¹ are decreased in PV-positive somas and at inhibitory presynaptic terminals in *Fgf14*^{-/-} animals. This phenotype is consistent with a diminished total pool of the two proteins,

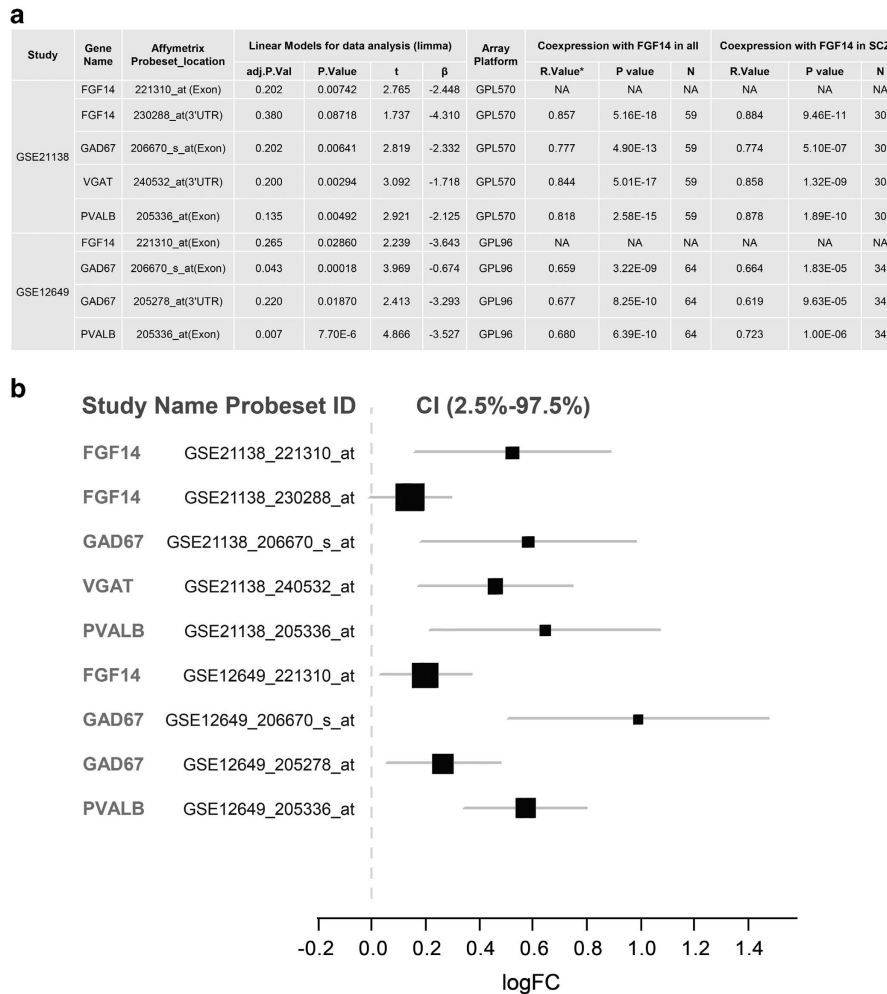


Figure 5. Differential gene expression and correlation of *FGF14*, *PVALB*, *VGAT* and *GAD67* in post-mortem control and schizophrenia samples. **(a)** The GSE21138 and GSE12649 data sets are both derived from previous studies^{60,61} and deposited in NCBI Gene Expression Omnibus (GEO). Owing to significant deviation from the mean (> 2 s.d.) in *FGF14* gene expression (221310_at), five samples were removed from the GSE12649 data set. GPL96 represents Affymetrix Human Genome U133A Array; GPL570 represents Affymetrix Human Genome U133 Plus 2.0 Array. GPL96 had no probeset selected for *VGAT* (SLC32A1). The original *P*-value was adjusted by Benjamini and Hochberg (false discovery rate). The *R*-value represents a Pearson Correlation with significance at the 0.01 level (two-tailed). **(b)** Forest plot illustrates the effect size (logFC) of differential gene expression of *FGF14*, *PVALB*, *VGAT* and *GAD67* between controls and schizophrenia patients in post-mortem dorsolateral prefrontal cortex (DLPFC) (BA46). Linear model and empirical Bayes method (Limma) was applied for assessing the differential gene expression of *FGF14* and its co-expression genes, including *PVALB*, *GAD67* and *VGAT*, in two independent data sets (GSE21138 and GSE12649) deposited in NCBI GEO. LogFC > 0 suggest decreased gene expression in patients with schizophrenia. The forest plot was created by R meta package. *VGAT*, vesicular gamma-amino-butyric acid transporter.⁶¹⁻⁶⁴

which might result from covariance of *FGF14*, *GAD67* and *VGAT* at expression level as suggested by our bioinformatics analysis from schizophrenia samples.

Whether loss of PV neurons and deficits in GABAergic markers in *Fgf14*^{-/-} brains occur through causative loops or are separate coincidental events remains to be determined. Evidence exists for two separate functions of *FGF14* as a regulator of intrinsic excitability at the AIS^{30,33,34,37,38,42,43} and a presynaptic organizer.^{40,41} Thus, phenotypes observed in *Fgf14*^{-/-} hippocampi might arise from disruption of two independent functions of *FGF14*: one impairing intrinsic firing of PV neurons, causing cell death, arrested development and/or aberrant circuitry integration, and one disrupting the presynaptic GABA machinery (synthesis and loading) via downregulation of selective markers (that is, *GAD67* and *VGAT*). Convergence of these disrupted functions might have fatal consequences for the final specifications and circuitry integration in PV neurons in the CA1 region. In an *Fgf14*

null condition, these uncompensated functions could be aggravated by concomitant loss of excitatory inputs, leading to a global remodeling of PV innervation fields,⁶⁹ shifting cortical networks into a high-PV status with reduced plasticity.⁶⁸

Given that the majority of GABAergic inputs in the CA1 area arise from PV interneurons, and that *GAD67* and *VGAT* are essential for the synthesis and loading of GABA at presynaptic terminals, we posited that *Fgf14* genetic deletion might impair hippocampal inhibitory transmission. Consistently, the distribution of sIPSC frequency (and amplitude) in *Fgf14*^{-/-} CA1 pyramidal neurons was found populated by longer inter-event intervals compared with *Fgf14*^{+/+} suggesting that in *Fgf14*^{-/-} mice the remaining inhibitory neurons might fire less than in control animals. sIPSCs are action potential-driven synaptic events and as such represent a compound readout of the firing status of presynaptic interneurons and the neurotransmitter release machinery. To examine GABA release mechanisms independent

of interneuron spontaneous firing, we isolated mIPSCs. The distributions of frequency and amplitude of mIPSCs in *Fgf14*^{-/-} mice were shifted with loss in short inter-event intervals and in smallest and largest amplitude events, respectively. These results highlight a combination of pre- and postsynaptic deficits in GABAergic transmission possibly induced by lower quantum content, probability of vesicle release or reduced number of vesicle, which might arise from structural and/or functional loss of a subset of inhibitory terminals. The lack in change of rise and decay time in the *Fgf14*^{-/-} mice argues against significant changes in the mechanism of diffusion of GABA across the synaptic cleft, or in the composition of postsynaptic ionotropic GABA receptors. However, high-resolution structural analysis is required for confirmation.

In cortical areas loss in PV neuron function and changes in GABAergic activity can desynchronize the E/I network leading to reduced gamma oscillations, a phenotype associated with schizophrenia and other psychiatric disorders.^{1,3,72} *In vivo* electroencephalogram local field potential recordings in the CA1 region revealed that in *Fgf14*^{-/-} animals the total, slow and fast, gamma band power was suppressed compared with control mice. These temporally segregated gamma oscillations reflect information carried by CA3 Schaffer collaterals and perforant path inputs, respectively.^{73–75} Thus, a reduction in both slow and fast gamma implies that both intra- and extra-hippocampal synaptic inputs might be compromised, possibly reflecting more widespread anomalies in *Fgf14*^{-/-} brains, arising from the prefrontal cortex–thalamic–hippocampal loop.⁷⁶ At the behavioral level, we show that *Fgf14*^{-/-} animals exhibit impaired spatial working memory, complementing the array of deficits commonly found in psychiatric disorders associated with cognitive impairment.⁵⁹

Human transcriptomics data confirmed functional clustering of *FGF14* with GABAergic signaling and identified a highly correlated decrease of *FGF14*, *PVALB*, *GAD67* and *VGAT* in schizophrenia post-mortem tissues, indicating possible genetic co-regulation of these genes. Thus, diminished expression of *FGF14* in humans might be a risk factor for complex brain diseases associated with cognitive impairment such as schizophrenia. These findings extend the original studies linking the dominant negative *FGF14*^{F1455} missense mutation to the inherited, rare disorder *SCA27* to a much broader set of human brain diseases.

The range of phenotypes from molecular to behavioral observed in *Fgf14*^{-/-} mice along with our corroborating human studies lay the groundwork for new mechanistic hypotheses on the biology and potential risk factors of cognitive impairment in schizophrenia and other complex brain disorders associated with E/I tone imbalance and disrupted development and plasticity of GABAergic signaling.⁷⁷ These findings further strengthen the emerging role of the AIS and its molecular components in the biology of diseases such as schizophrenia, bipolar disorder and depression.⁴²

CONFLICT OF INTEREST

HYM has served as a consultant for Forum, Lundbeck, Reviva and Sunovion; he owns stocks in Acadia Pharmaceuticals, GlaxoSmithKline and SureGene. The remaining authors declare no conflict of interest.

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