



Interneuron Heterotopia in the *Lis1* Mutant Mouse Cortex Underlies a Structural and Functional Schizophrenia-Like Phenotype

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LIS1 is one of the principal genes related to Type I lissencephaly, a severe human brain malformation characterized by an abnormal neuronal migration in the cortex during embryonic development. This is clinically associated with epilepsy and cerebral palsy in severe cases, as well as a predisposition to developing mental disorders, in cases with a mild phenotype. Although genetic variations in the *LIS1* gene have been associated with the development of schizophrenia, little is known about the underlying neurobiological mechanisms. We have studied how the *Lis1* gene might cause deficits associated with the pathophysiology of schizophrenia using the *Lis1/sLis1* murine model, which involves the deletion of the first coding exon of the *Lis1* gene. Homozygous mice are not viable, but heterozygous animals present abnormal neuronal morphology, cortical dysplasia, and enhanced cortical excitability. We have observed reduced number of cells expressing GABA-synthesizing enzyme glutamic acid decarboxylase 67 (GAD67) in the hippocampus and the anterior cingulate area, as well as fewer parvalbumin-expressing cells in the anterior cingulate cortex in *Lis1/sLis1* mutants compared to control mice. The cFOS protein expression (indicative of neuronal activity) in *Lis1/sLis1* mice was higher in the medial prefrontal (mPFC), perirhinal (PERI), entorhinal (ENT), ectothalamic (ECT) cortices, and hippocampus compared to control mice. Our results suggest that deleting the first coding exon of the *Lis1* gene might cause cortical anomalies associated with the pathophysiology of schizophrenia.

Keywords: *LIS1* gene, schizophrenia, GABAergic system, interneurons, hippocampus, mPFC, c-fos

INTRODUCTION

Abnormal neuronal migration during brain development results in several brain organization alterations. One of the most significant cortical abnormalities is lissencephaly (Reiner et al., 1995; Kato and Dobyns, 2003; Barkovich et al., 2005; Wynshaw-Boris, 2007). This developmental alteration produces severe symptoms that include seizures, intellectual retardation, and a higher risk of developing psychotic disorders like schizophrenia (Dobyns et al., 1993; Tabarés-Seisdedos et al., 2008). Schizophrenia (SZ) is a chronic and serious psychiatric illness that occurs in 1% of

the global population (Freedman, 2003) and which is caused by both genetic and environmental factors. The symptoms of SZ fall into three categories: (1) positive symptoms that include hallucinations, delusions, cognitive deficits, and movement disorders; (2) negative symptoms, such as the disruption of normal emotions and behaviors; and (3) cognitive symptoms: attention deficit and memory problems (Ross et al., 2006; Insel, 2010).

The precise cause of SZ remains unclear, postmortem studies have shown a decrease of GABA-synthesizing enzyme (GAD67) together with a reduction in the number of parvalbumin (PV)+ interneurons in the medial prefrontal cortex (mPFC) (Hashimoto et al., 2003, 2008; Reynolds et al., 2004; Woo et al., 2008; Glausier et al., 2014; Hoftman et al., 2015). Other GABAergic subtypes of interneurons, such as cholecystokinin, vasoactive intestinal peptide, somatostatin, neuropeptide Y, and calbindin (CB) have also been reported to be less expressed in the mPFC of SZ patients (Hashimoto et al., 2003, 2008; Fung et al., 2010, 2014). These deficits in the GABAergic interneurons seem to be related to the alteration of PFC-dependent cognitive functions in SZ patients. Indeed, PFC is implicated in important cognitive processes such as attention, memory, and behavioral changes (Heidbreder and Groenewegen, 2003; Gabbott et al., 2005; Briand et al., 2007).

Furthermore, functional failures of N-methyl-D-aspartate (NMDA) receptors, typically found in GABAergic neurons, affect the maturation of GABAergic interneurons (Thomases et al., 2013). Interestingly, previous studies provided evidence that NMDA receptor antagonists, such as ketamine, MK-801, and phencyclidine (PCP), reproduce schizophrenia-like symptoms in mice. For this reason, blocking NMDA receptors is the approach most widely used to mimic SZ symptoms in animal models (Jentsch and Roth, 1999; Ozdemir et al., 2012; Gilabert-Juan et al., 2013; Kim et al., 2014; Li J.-T. et al., 2016; Castillo-Gómez et al., 2017). In mice, PCP treatments generate abnormal behaviors (Boyce et al., 1991), including hyperactivity, stereotypic behaviors, and motor dysfunction (Castellani and Adams, 1981; Nabeshima et al., 1987). Accordingly, PCP produces schizophrenia-like psychosis, and increases psychotic symptoms in SZ patients (Javitt and Zukin, 1991). Moreover, several studies have reported that PCP induces cFOS protein expression in pontine nuclei and the thalamus, as well as in cortical regions including the retrosplenial, pyriform, cingulate, and frontal cortices. These results suggest a potential relationship between increased cFOS and the psychotic effects of the NMDA receptor antagonists (Abdel-Naby Sayed et al., 2001).

The LIS1 complex is a tetramer intracellular enzyme composed of two catalytic subunits, PAFAH-1B3 (ALPHA1) and PAFAH-1B2 (ALPHA2), and a regulatory dimer of PAFAH1B1 (LIS1) (Hanahan, 1986; Kornecki and Ehrlich, 1988; Koltai et al., 1991a,b; Chao and Olson, 1993; Stafforini et al., 2003; Bazan, 2005; Escamez et al., 2012). *Lis1* is a gene strongly expressed in the cortical plate during embryogenesis and at postnatal stages in the hippocampus, neocortex and cerebellum (Reiner et al., 1995). Genetic variations in the lissencephalic critical region (17p13.3) around *LIS1* gene, have been observed in schizophrenia patients.

Previous studies of our group have shown that patients showed a greater tendency toward genetic variations of *Lis1* markers (Tabarés-Seisdedos et al., 2006, 2008). Moreover, variations of *LIS1* expression have been reported in the dorsolateral prefrontal cortex of patients with schizophrenia (Lipska et al., 2006) and functional alterations of LIS1 protein interactive complex in brain development are relevant to the pathogenesis of schizophrenia and related psychiatric illness (Bradshaw et al., 2011).

In this study, we analyzed the effect that deleting the first coding exon of the *Lis1* gene has on the distribution of GABAergic interneurons in the young-adult stages of *Lis1/sLis1* mutant mice. We found a reduction of GABAergic interneurons in both the hippocampus and Anterior Cingulate area (ACA) in *Lis1/sLis1* mutant mice compared to control animals. In addition, the immunohistochemistry of the cFOS protein was analyzed to evaluate neuronal activity in different brain areas. Furthermore, we explored the activation of GABAergic interneurons in *Lis1/sLis1* mice. To do this, we analyzed the expression of cFOS in GABAergic interneurons in cortical regions and the hippocampus. Finally, behavioral studies demonstrate the dysfunction of mutant mice in terms of spontaneous locomotion and cognitive tests.

Our results suggest that this mutation in the *Lis1* gene could cause changes associated with some SZ endophenotypes.

MATERIALS AND METHODS

Animals

All mutant *Lis1/sLis1* mice had an ICR genetic background. The *Lis1/sLis1* transgenic line has been described previously (Cahana et al., 2001). For genotyping, PCR was performed as described in Cahana et al. (2001), using one set of primers to detect the gene codifying the shorter protein (sLIS1): Mlis1 5'-GGT GGC AGT GTT GAG ATG CCT AGC C-3' and Mlis1 5'-GCA TTC CTG TAA TCC AGT ACC TGG-3' (**Supplementary Figure 1**). The 67-green fluorescent protein (*Gad67-Gfp*) line was described by Tamamaki et al., 2003. The *Gad67-Gfp* × *Lis1/sLis1* strain was generated by crossing heterozygous *Lis1/sLis1* mice with *Gad67-Gfp* mice.

The animal experiments complied with the Spanish and European Union laws on animal care in experimentation (Council Directive 86/609/EEC). To stage the animals, we took the vaginal plug formation date as embryonic day 0.5 (E0.5). To fix adult brains from postnatal day 30 (P30) or 60 (P60) mice, the animals were anesthetized with isoflurane (Zoetis, United Kingdom), perfused transcardially with phosphate-buffered saline (PBS, pH7.4), followed by 4% paraformaldehyde (PFA) in PBS. The brains were subsequently removed and postfixed overnight at 4°C in the same fixative.

Some animals were selected for P60 behavior tests. An equal number of males and females were used in each experiment.

Immunohistochemical Staining

The animals were processed for immunohistochemistry staining to quantify the three subtypes of GABAergic interneurons (PV+, CB+, and CR+ cells), glutamic acid decarboxylase

(GAD67) and cFOS. After perfusion, the brains were removed, postfixed in the same fixative overnight and embedded in 4% agarose in PBS, and transversely sectioned into 70 μm -thick sections using a Vibratome (Leica). The brain slices were then rinsed with phosphate buffer solution containing 0.075% Triton X-100 (PBST) and processed for immunohistochemistry staining. For cFOS immunohistochemistry, the brains were soaked in 10, 20, and 30% sucrose solution at 4°C in PBS, and coronal sections (40- μm thick) were cut using a cryostat (HM525, Microm/Thermo Fisher Scientific, Waltham, MA, United States). The vibratome sections were incubated with 0.09% hydrogen peroxide (H_2O_2) for 30 min, rinsed with phosphate buffer solution containing 0.075% Triton X-100 (PBS-T). Next, the tissue was incubated with Goat Serum (GS) (blocking solution) for 1 h to avoid any non-specific antigen binding. The sections were then incubated overnight at 4°C with the proper primary antibodies diluted in EnVision FLEX Antibody Diluent (DAKO, Denmark). The antibodies used were: anti-green fluorescent protein (GFP) chicken polyclonal antibody (1:500; Aves Labs, Inc., Tigard, Oregon); anti-calbindin D-28k (CB) rabbit polyclonal antibody (1:2000; Swant, Bellizona, Switzerland); anti-calretinin (CR) rabbit polyclonal antibody (1:2000; Swant, Bellizona, Switzerland); anti-parvalbumin (PV) polyclonal antibody (1:2000; Swant, Bellizona, Switzerland); and anti-cFOS rabbit polyclonal IgG (1:400; Calbiochem). The next day, the sections were rinsed three times at room temperature and incubated with the biotinylated secondary antibody for 1 h.

Afterward, the sections were washed with PBS-T and incubated with Avidin-Biotin Complex for 1 h (1:300; ABC kit, Vector Laboratories CA-94010). For the colorimetric detection (black), the tissue was incubated with 1% 3,3'-Diaminobenzidine (DAB; Vector Laboratories SK-4100), 0.025% ammonium nickel sulfate hexahydrate, and 0.0018% H_2O_2 in PBS).

In some cases, we performed double immunohistochemistry for anti-PV and anti-vesicular glutamate transporter 1 (VGLUT1) polyclonal guinea pig antibody (1:4000; Millipore, Temecula, CA, United States) or anti-cFOS rabbit and anti-GFP. For this, the samples were processed for immunofluorescence staining (free Triton X-100). For this immunostaining, the sections were incubated with the appropriate secondary antibody for 1 h, after being incubated overnight with either anti-parvalbumin and anti-VGLUT1 or anti-cFOS and anti-GFP. The secondary antibodies used were: Alexa Fluor 488 donkey anti-rabbit IgG 1:500 (Molecular Probes/Invitrogen#A11055) and Alexa Fluor 488 goat anti-chicken IgG (A11039/Molecular Probes), and donkey anti-guinea pig IgG 1:200 (Biotium, Jackson Immuno Research) or goat anti-rabbit IgG 1:300 (BA-9200/Vector) followed by Cy3-Streptavidin 1:500 (PA43001/Amersham).

Next, the sections were washed with PBS-T RT and the nuclei were counterstained with 4',6-diamidino-2-phenylindole (DAPI, Molecular Probes/Invitrogen) diluted 1:1000 in PBS for 10 min and the sections were then washed in PBS. Finally, the sections were mounted on glass slides using 10:1 Mowiol (Calbiochem)-NPG (Sigma).

Microscopy and Statistical Analysis

Images were acquired using optical (Leica CTR6000) and confocal (Leica TCS SPE) microscopes, and the images were used to make immunopositive cell counts. To quantify the number of cells, we counted four sections per animal, in a parallel series of sections stained using different markers; we used Image-J software (NIH, United States) for cell counting. The data used for statistical analysis was the mean of the four sections counted. To quantify VGLUT1+ staining on PV-interneurons we selected two sections and five interneurons in each section (see **Figure 3** below). On the GFP+ channel (PV+ neurons) we chose a segment of a dendrite in each selected neuron, and then we measured the area of that segment and the area of red boutons/clusters placed in contact with that dendrite segment. The total area of the dendritic segments selected was the same in WT and mutant sections and we measured the % of dendritic area covered by red stained boutons; these measurements were made with Image-J in a blind manner.

We focused on three mPFC subregions: the ACA, the prelimbic cortex (PrL) and infralimbic cortex (IL); we studied also the hippocampal region and the perirhinal (PERI), entorhinal (ENT), ectorhinal (ECT) cortices in both the right and left hemispheres using at least four different sections for each brain area. To identify these regions, the Allen Brain Atlas¹ was used as a guide. The average of these determinations for each section was defined as the number of immunopositive cells within a specified area in the individual brain. The average of these values among different sections of an individual brain was used for statistical analysis. In all cases, the data are expressed as the mean \pm standard error of the mean (SE). Statistical differences between *Lis1/sLis1* mice and control littermates were determined using the Student's *t*-test. The Mann-Whitney *U* test was used to compare differences between two independent groups when the dependent variable was not normally distributed or when their variances were not equal. To perform statistical analysis Sigma Plot software was used. A value of $p < 0.05$ was considered statistically significant. Significance levels were set to: * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$.

Behavioral Tests

Locomotor Activity

For the Open Field Test, the spontaneous activity of the mice was assessed by monitoring their activity in an open field (SMART VIDEO TRACKING Software, Panlab, Barcelona, Spain). The apparatus consisted of a square plexiglass box (50 cm wide \times 50 cm long \times 40 cm high). The mice (P60) were placed, individually, in the center of the apparatus, and left undisturbed for 10 min. The cage was thoroughly cleaned with 75% ethanol between each test to eliminate any olfactory cues. The total ambulatory distance was analyzed.

To record voluntary wheel-running activity, each cage was equipped with a running wheel (Med Associates Inc., Vermont, United States) at P60. The animals had constant voluntary access to the running wheel. The data was recorded with a standard computer using the Wireless Running Wheel Interface System

¹<http://atlas.brain-map.org/>

(Med Associates Inc., Vermont, United States). The number of wheel revolutions each minute were recorded over a 72-h period. From the raw data, the total revolutions, day/night revolutions, and the 12-h revolution average were calculated using Excel 2016 (Microsoft Corporation, Washington, United States).

Novel Object Recognition Test

The Novel Object Recognition Test (NOR) ($n = 12$ for each group; P60) was used to assess non-spatial memory in a box (50 cm wide \times 50 cm long \times 40 cm high). After a 30 min habituation period the previous day, the behavior was recorded using a camera equipped with a computer-assisted data acquisition system (Smart, PANLAB, Spain), in both training and retention sessions. Two novel objects were symmetrically placed 15 cm from the walls during the training session. Each mouse explored for 5 min. Exploration of the objects was defined by the time a mouse spent with its head facing the objects within 1 cm of them, or if it was touching or sniffing them. The objects and box were cleaned with 75% ethanol after the training session. The retention tests were carried out 5 min after the training session, and one of the objects used during training was replaced with a novel object. Each mouse was then allowed to freely explore for 5 min. Exploratory preference was calculated by the ratio of the time spent exploring the novel object over the total time spent exploring the two objects.

RESULTS

GABAergic Signaling

Data published to date has revealed the potential role of cortical GABAergic deficits in the symptoms and pathology observed in SZ patients (Lewis et al., 2012; Nakazawa et al., 2012; Stansfield et al., 2015; Li J.-T. et al., 2016). To study the GABAergic system in *Lis1/sLis1* mutant mice, we quantified the number of PV, CR, CB and GAD67-positive neurons in the mPFC and hippocampus (Hi) of mice brains at 30 days (P30).

Reduction of Number of Cells Expressing GAD67 Is Observed in the Anterior Cingulate Area and Hippocampus in *Lis1/sLis1* Mutant Mice

The number of cells expressing GABA-synthesizing enzyme glutamic acid decarboxylase 67 (GAD67), responsible for most GABA synthesis, was studied in *Lis1/sLis1* mutant and control mice by using the mutant strain *Gad67-Gfp* \times *Lis1/sLis1*. GAD67-positive cells were detected through GFP immunohistochemistry at P30. The distribution of GAD67+ interneurons was studied in the hippocampus and mPFC (Figure 1). A decrease in the number of interneurons expressing this enzyme was observed in the hippocampus of the *Lis1/sLis1* mutant mice (Figures 1A–E). GAD67 reactivity was noted in scattered cells of all the CA1–CA3 layers and, occasionally, in cells of the dentate gyrus (DG) in control mice (Figures 1A,C). The analysis of the *Lis1/sLis1* mutant hippocampus showed that the number of cells expressing GAD67 was reduced in the entire hippocampal area (Figures 1B,D; $n = 4$; controls, 705 ± 28 ; *Lis1/sLis1*, 623 ± 26 ; $p < 0.05$, Student's *t*-test). These differences become more evident

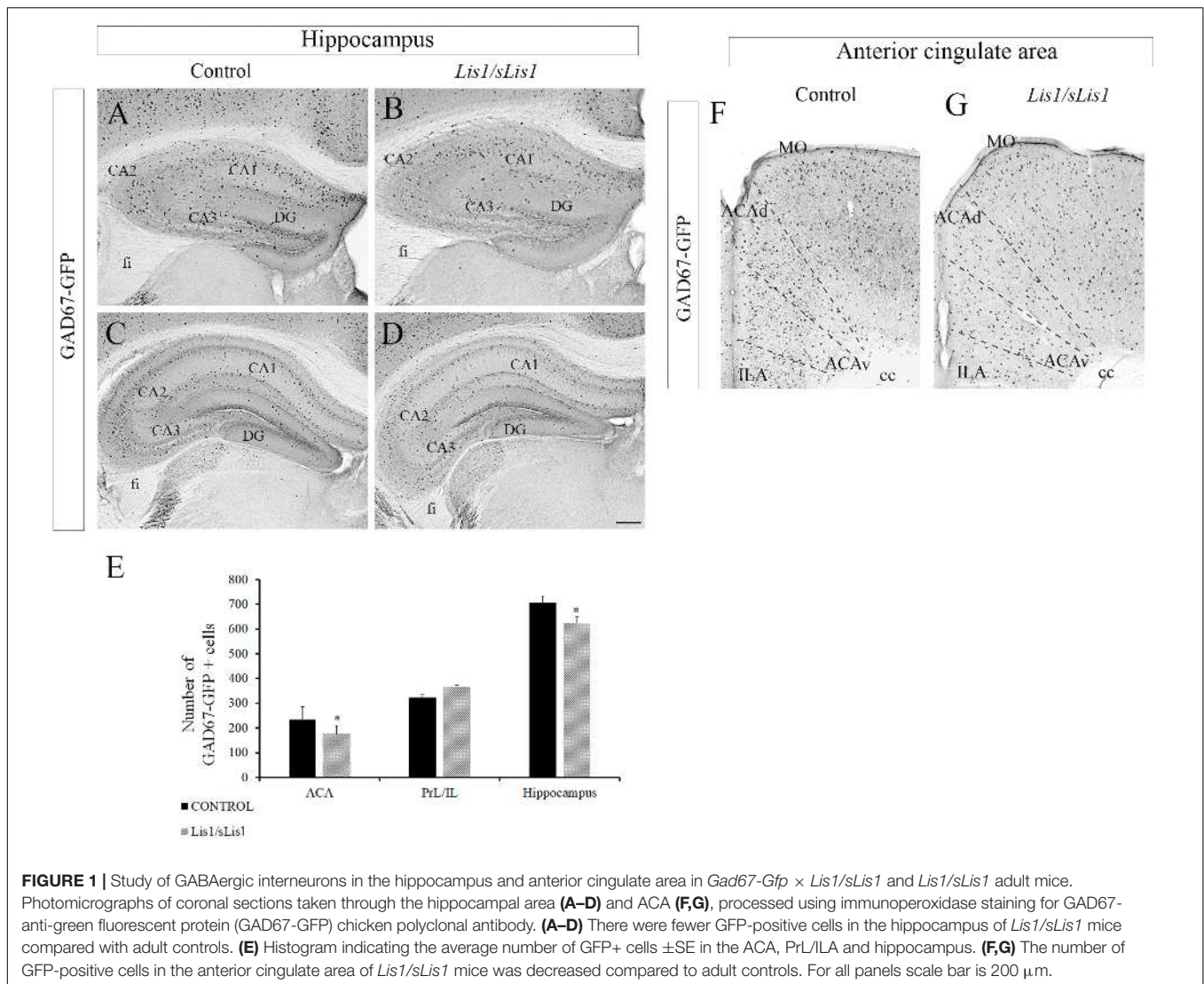
in the CA3 area, where a severe reduction of GAD67+ cells was observed (Figure 1D). The mPFC is subdivided into three main parts: the anterior cingulate (ACA), prelimbic (PrL), and infralimbic (IL) areas (Tavares and Corrêa, 2006; Vertes, 2006). Although no differences were observed in the number of GAD67-positive interneurons in the infralimbic (IL) and prelimbic (PrL) areas, a reduction of the number GAD67+ interneurons was detected in the ACA ($n = 4$; Figures 1F–H; $n = 4$; controls, 235 ± 50 ; *Lis1/sLis1*, 176 ± 32 ; $p < 0.05$, Student's *t*-test).

Fewer PV+ Cells in the Anterior Cingulate Area of *Lis1/sLis1* Mutant Mice

Alterations of PV-expressing interneurons have been implicated in many neuro-psychiatric diseases, including schizophrenia (Flames et al., 2004; Fisahn et al., 2009; Fazzari et al., 2010; Ting et al., 2011; Huang et al., 2021). Accordingly, we analyzed the distribution of PV-positive interneurons in *Lis1/sLis1* mutant and control mice in the PrL, IA, and ACA areas (Figure 2). No differences were observed in the number of PV-positive interneurons in the PrL and IL areas ($n = 4$, average number of PV-positive interneurons \pm SE: controls, 63.9 ± 7.54 ; *Lis1/sLis1*, 68.9 ± 3.28). We detected a significant decrease of PV interneurons in the ACA of *Lis1/sLis1* mutant mice compared to control animals ($n = 4$; Figures 2A,B; $n = 4$; average number of PV+ neurons \pm SE: controls, 103 ± 3.30 ; *Lis1/sLis1*, 65 ± 8.70 ; $p < 0.01$, Student's *t*-test). That reduction of PV+ cells was prominent in layers 2/3 and 5, where they are most abundant, whereas differences in layer six were less marked (Figure 2B). The values shown correspond to the mean \pm SE ($n = 4$; Figure 2E). No significant differences in PV interneurons were observed in the hippocampus ($n = 4$, average number of PV-positive interneurons \pm SEM: controls, 104 ± 6.77 ; *Lis1/sLis1*, 110 ± 6.15).

Decreased CR+ Cells in the Anterior Cingulate Area of *Lis1/sLis1* Mutant Mice

As a continuation of the study of GABAergic populations of interneurons, we examined the distribution of CR and CB positive interneurons in the mPFC of control and *Lis1/sLis1* mutant mice. In control mice, we observed CR+ interneurons distributed throughout the ACA, most frequently being found in layer 2/3, whereas in layers 5 and 6 less CR-positive interneurons were marked (Figures 2C,D). In accordance with our previous results on PV interneurons, we observed no differences in the number of CR-positive interneurons in the PrL and IL areas of the mPFC ($n = 4$, average number of CR-positive interneurons \pm SE: controls, 107 ± 4.05 ; *Lis1/sLis1*, 98 ± 5.46). Nevertheless, a decrease in CR interneurons was observed in ACA in *Lis1/sLis1* mutant mice (Figures 2C,D). The values shown correspond to the mean \pm SE ($n = 4$; Figure 2E; $n = 4$ average number of CR+ neurons \pm SE: controls, 134 ± 18 ; *Lis1/sLis1*, 84 ± 4 ; $p < 0.01$, Student's *t*-test). No significant differences were observed for CB-positive interneurons in any of the mPFC regions ($n = 4$, average number of CR-positive interneurons \pm SE: controls, 114 ± 8.05 ; *Lis1/sLis1*, 112 ± 9.70). The analysis of the hippocampus in *Lis1/sLis1* mutant mice revealed no differences in CB- or CR-expressing interneurons between mutant and control mice ($n = 4$,



average number of CR-positive interneurons ± SE: controls, 109 ± 8.50 ; *Lis1/sLis1*, 105 ± 8.72 ; $n = 4$, average number of CB-positive interneurons ± SE: controls, 163 ± 32.29 ; *Lis1/sLis1*, 169 ± 17.60).

Decreased VGLUT1+ Terminals Contacting PV+ Interneurons in the Hippocampus of *Lis1/sLis1* Mutant Mice

Previous studies have reported decreased excitatory terminals in cortical interneurons in SZ patients (Chung et al., 2016) as well as in mouse models displaying a SZ-like phenotype (Del Pino et al., 2013). The reduced number of PV+ interneurons and the lowered excitatory drive to PV+ cells (Coyle et al., 2012; Gonzalez-Burgos et al., 2015; Chung et al., 2016) have been proposed as the neural substrate for cognitive dysfunction in SZ. Therefore, we first analyzed the distribution of PV-positive interneurons in the hippocampal areas of *Lis1/sLis1* mutant and control mice. As we have described above, no differences were observed in the number of PV-positive interneurons in

the hippocampus. Next, we quantified the excitatory synapses contacting PV+ interneurons. To do this, we stained the vesicular glutamate transporter 1 (VGLUT1) by immunohistochemistry to detect excitatory presynaptic contacts with PV+ interneurons in *Lis1/sLis1* mutant mice and in their control littermates. We quantified the area of VGLUT1 boutons contacting PV+ interneurons in the hippocampal region and in the mPFC of *Lis1/sLis1* mutant and control mice, using the Image J software. We observed a decrease in the area covered by VGLUT1 boutons in PV+ cells in both the CA1-3 and DG regions of *Lis1/sLis1* mutants compared to controls (Figures 3A–C; $n = 4$, average of% of PV+ dendrite opposed to VGLUT1 ± SE: controls $21.3 \pm 2.1\%$; *Lis1/sLis1*, $14.6 \pm 2.6\%$, $p < 0.05$, Student's *t*-test). However, no significant differences were observed in the mPFC ($n = 4$, average of% of PV+ dendrite opposed to VGLUT1 ± SE: controls $19.7\% \pm 2.3\%$; *Lis1/sLis1*, $22.9\% \pm 2.6\%$). These results demonstrate that hippocampal PV interneurons of CA1-3 and DG receive a reduced number of excitatory synapses in *Lis1/sLis1* mutant mice.

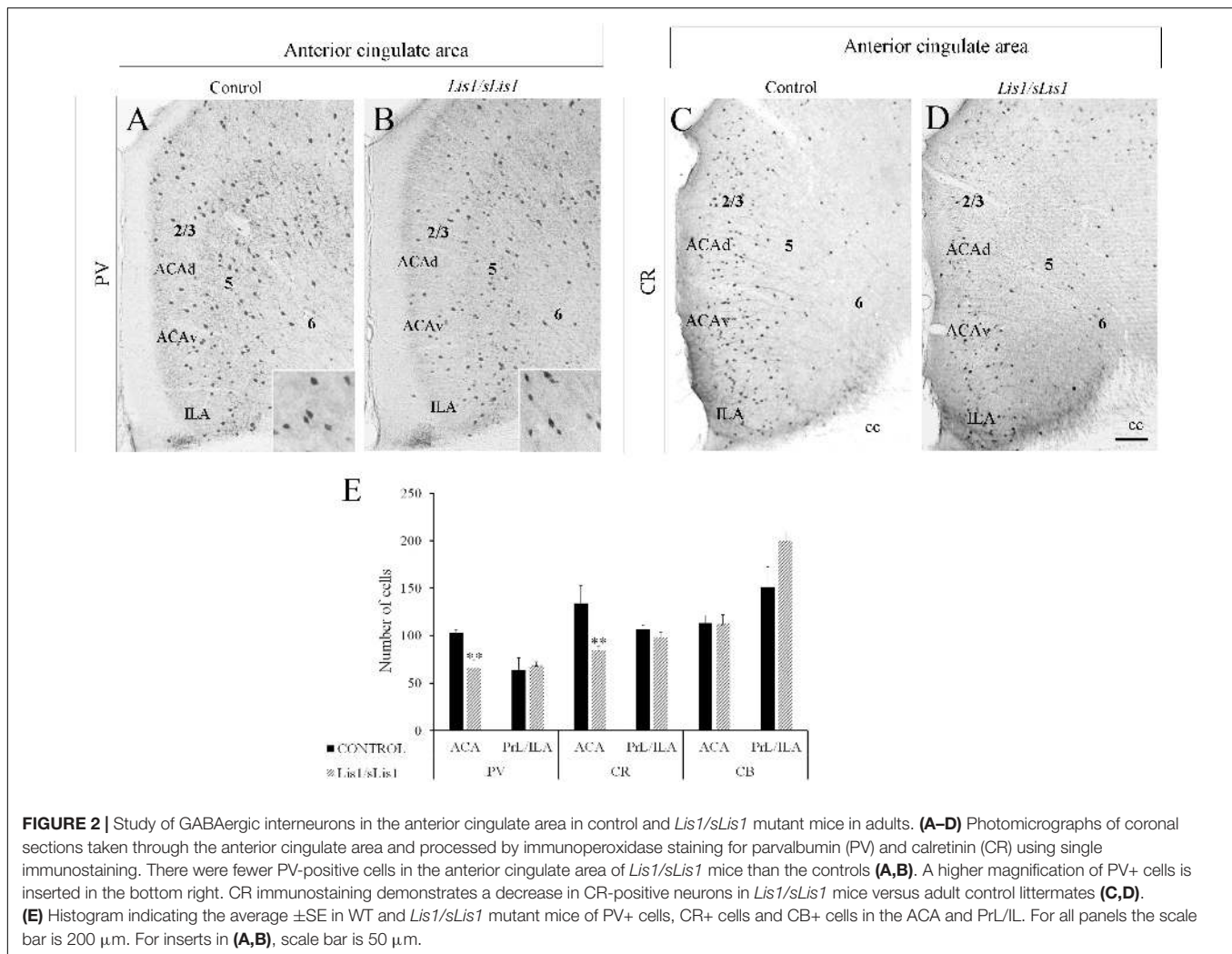


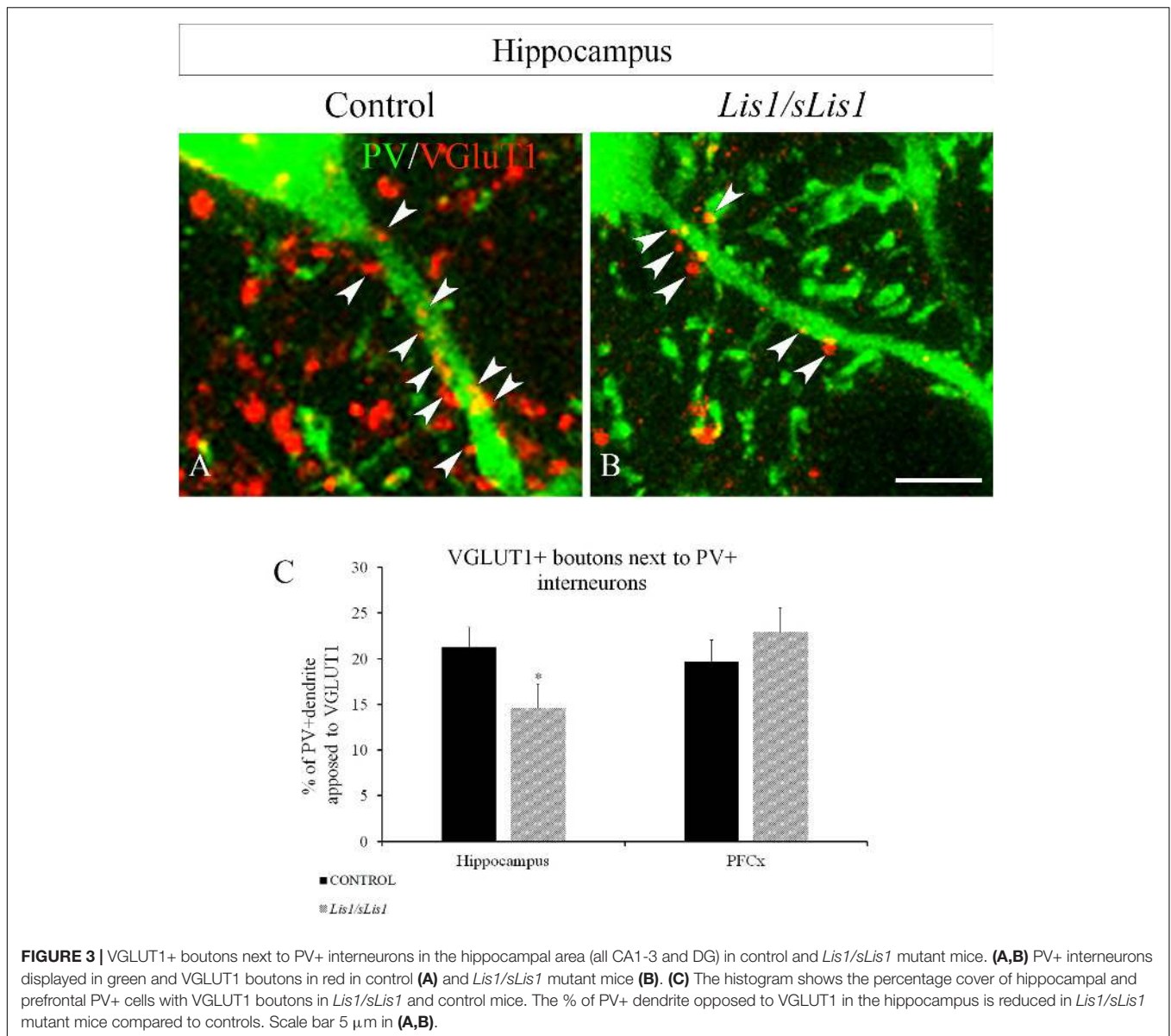
FIGURE 2 | Study of GABAergic interneurons in the anterior cingulate area in control and *Lis1/sLis1* mutant mice in adults. **(A–D)** Photomicrographs of coronal sections taken through the anterior cingulate area and processed by immunoperoxidase staining for parvalbumin (PV) and calretinin (CR) using single immunostaining. There were fewer PV-positive cells in the anterior cingulate area of *Lis1/sLis1* mice than the controls **(A,B)**. A higher magnification of PV+ cells is inserted in the bottom right. CR immunostaining demonstrates a decrease in CR-positive neurons in *Lis1/sLis1* mice versus adult control littermates **(C,D)**. **(E)** Histogram indicating the average \pm SE in WT and *Lis1/sLis1* mutant mice of PV+ cells, CR+ cells and CB+ cells in the ACA and PrL/IL. For all panels the scale bar is 200 μ m. For inserts in **(A,B)**, scale bar is 50 μ m.

Increased Number of Positive Cells for cFOS in the mPFC, Hippocampus and Other Related Cortical Areas in *Lis1/sLis1* Mutant Mice

Proteins coded by immediate-early genes such as *cFOS*, are considered markers of neuronal activity in the central nervous system (Sagar et al., 1988; Herdegen et al., 1995; Kontkanen et al., 2002). Moreover, it has been established that the number of *cFOS*-expressing neurons increases in animal models of schizophrenia (Celada et al., 2013; Hervig et al., 2016; Calovi et al., 2020). Here, we analyzed the distribution pattern of *cFOS* immunopositive cells in the hippocampal areas, mPFC, PERI, ECT, and ENT of both control and *Lis1/sLis1* mutant mice. To this end, we quantified the number of cells expressing *cFOS*. We counted *cFOS* positive cells at P60 in control and *Lis1/sLis1* mutant mice that had been exposed to the same basal environmental stimuli in the animal facility (face-to-face cages in the same room). In the hippocampus of control young-adult mice most of the *cFOS* protein was expressed in the granular cell layer of the DG and the pyramidal cell layer of CA1–CA3. *cFOS* was also detected, but at lower densities, in the DG molecular layer (hilus), and

in the stratum oriens of the CA1–CA3 (Figures 4A,C,E). We detected a significant increase in *cFOS* immunopositive cells in CA1–CA3 regions (Figures 4B,D), as well as in the dentate gyrus (Figure 4E) in the hippocampus of *Lis1/sLis1* mutant mice compared to control mice ($n = 4$; average number of *cFOS*+ cells in CA \pm SE: controls, 359 ± 20 , $n = 4$, *Lis1/sLis1*, 498 ± 22 , $n = 4$; $p < 0.01$, Student's *t*-test; average number of *cFOS*+ cells in DG \pm SE: controls, 45 ± 4 , $n = 4$, *Lis1/sLis1*, 148 ± 9 , $n = 4$; $p < 0.001$, Student's *t*-test). Moreover, we analyzed cells positive for *cFOS* in both the suprapyramidal (DGsp) and infrapyramidal (DGip) blades of the DG. In control mice, *cFOS* positive cells were mainly observed in the DGsp, whereas fewer *cFOS* positive cells were seen in the DGip (Figure 4E), suggesting a different expression pattern of *cFOS* protein in the two DG blades. Strikingly, this difference in neuronal activity between DGsp and DGip was not observed in the DG of *Lis1/sLis1* mice. The DGip of the DG in *Lis1/sLis1* mutant mice there was a greater number of cells expressing *cFOS* compared to the control mice (Figures 4E,F,I).

Subsequently, we analyzed *cFOS*-positive cells in the mPFC and observed that this area also presented more *cFOS*-positive cells in *Lis1/sLis1* mice than the control animals (Figure 4G–I;

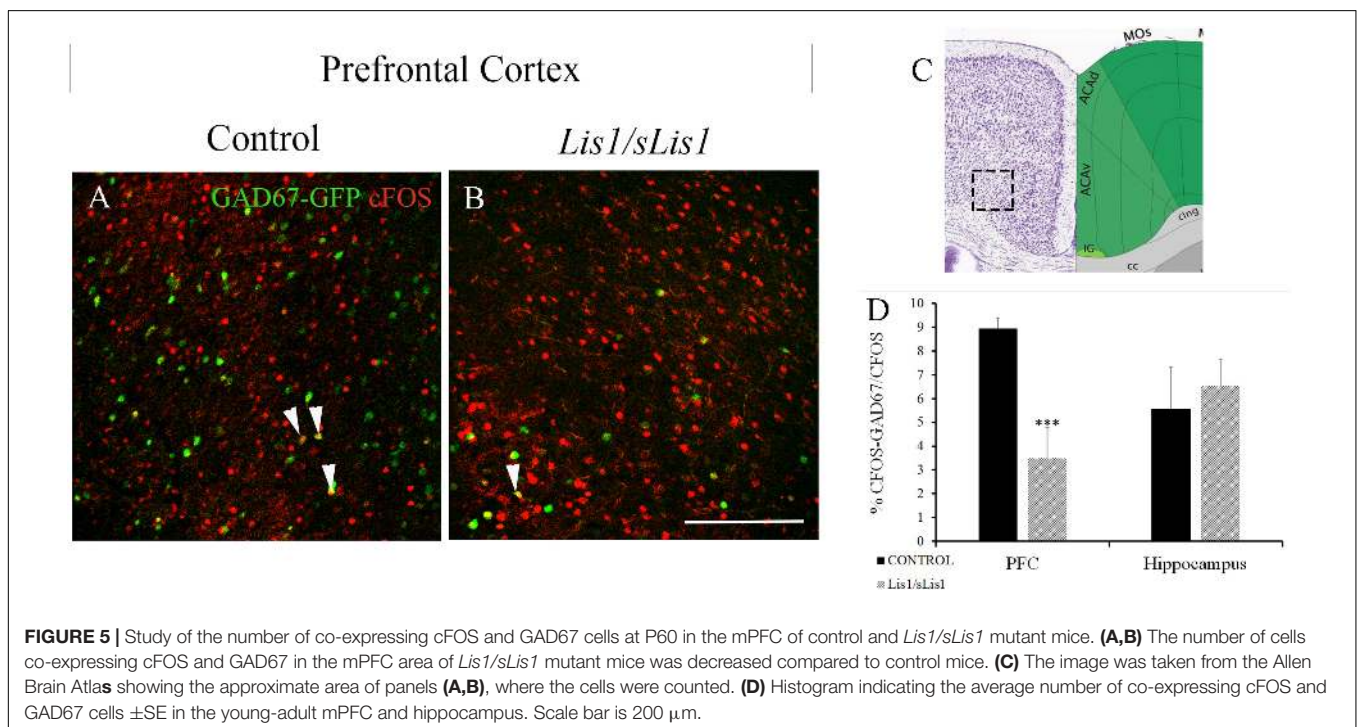
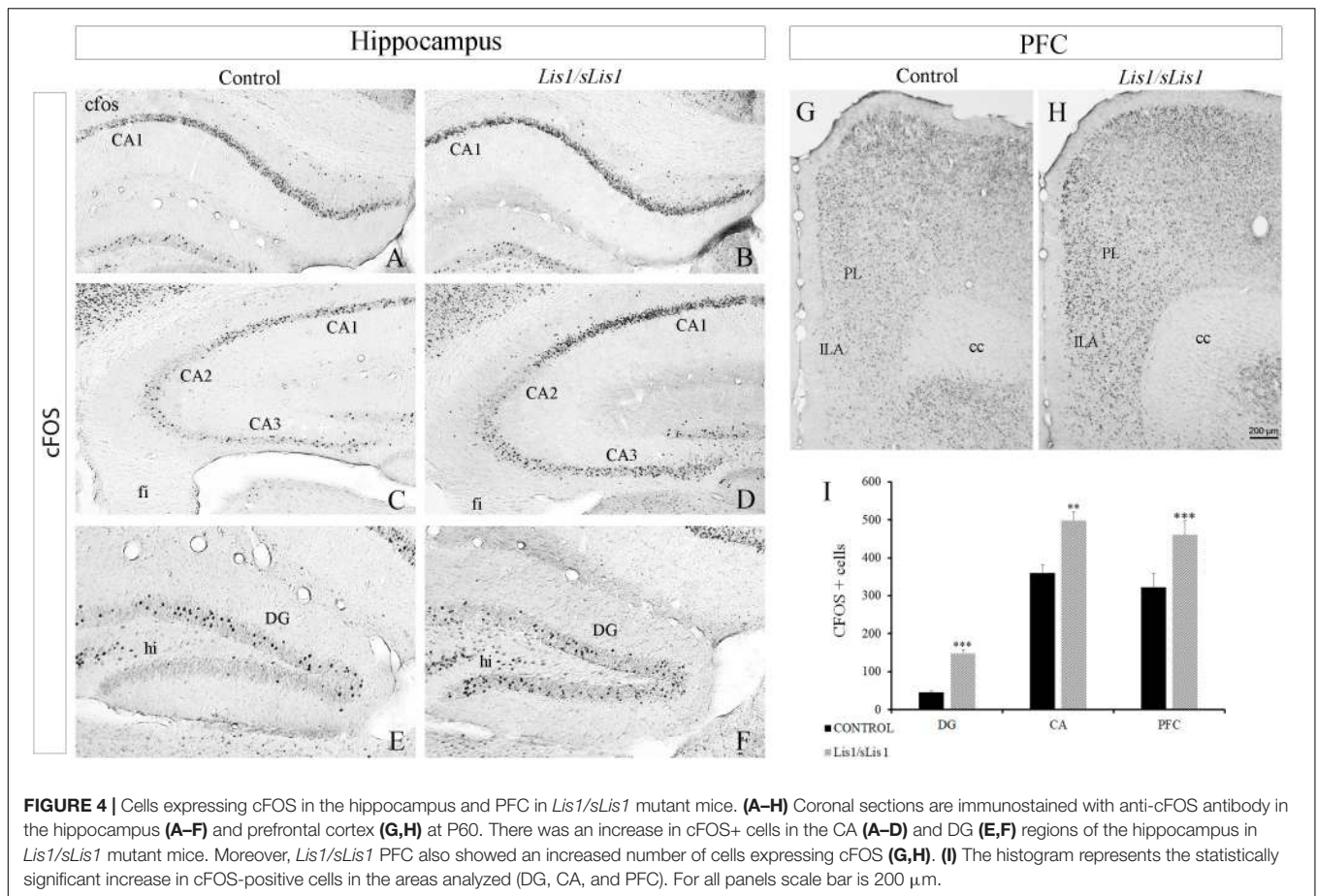


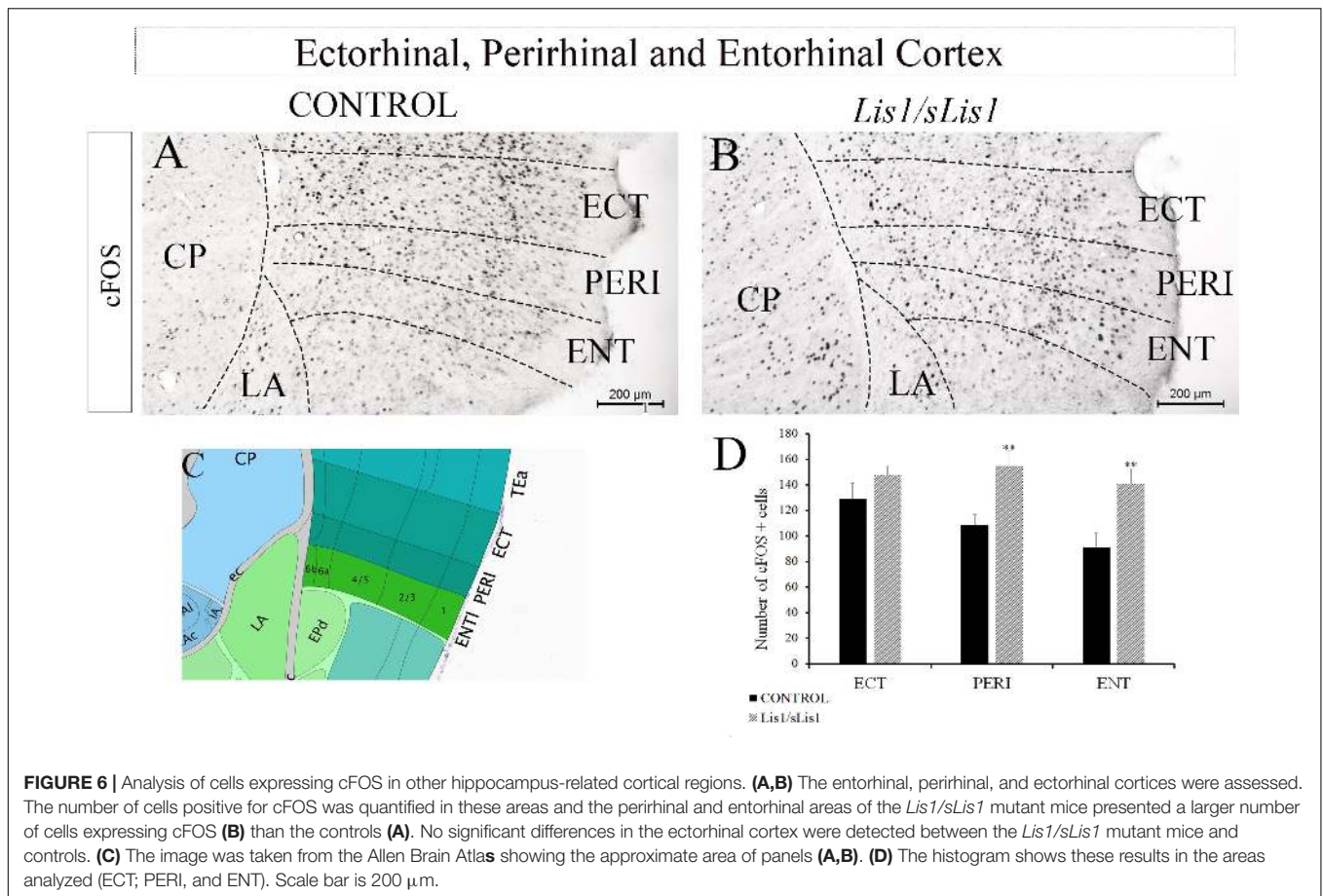
$n = 4$; average number of cFOS+ cells in mPFC \pm SE: controls, 322 ± 36 , $n = 4$, *Lis1/sLis1*, 460 ± 37 , $n = 4$; $p < 0.001$, Student's *t*-test).

In addition, we analyzed the neuronal activity of GAD67+ interneurons. To this end, we quantified the number of cells co-expressing cFOS and GAD67 at P60 in control and *Lis1/sLis1* mutant mice (Figure 5). We analyzed the number of GAD67+ interneurons that were immunopositive for cFOS in both the mPFC and hippocampal areas. We detected fewer cells co-expressing cFOS and GAD67 with respect to the total number of cFOS positive cells, in the mPFC area of *Lis1/sLis1* mutant mice compared to control mice (Figures 5A–D; $n = 4$; average number of % cFOS-GAD67/cFOS cells \pm SE: controls, 8.95 ± 0.45 ; *Lis1/sLis1*, 3.48 ± 1.28 , $p < 0.001$, Student's *t*-test). No significant differences were observed in the number of cells co-expressing cFOS and GAD67 when the hippocampus was analyzed ($n = 4$,

average number of % cFOS-GAD67/cFOS cells \pm SE: controls, 5.56 ± 1.79 ; *Lis1/sLis1*, 6.56 ± 1.11). However, when the number of cells co-expressing cFOS and GAD67 with respect to the total number of GAD67 positive cells was studied in the mPFC area of *Lis1/sLis1* mutant mice compared to control mice, no significant differences were detected suggesting that interneuron excitability remains intact.

Hippocampal functions such as episodic memory and spatial orientation, are associated with other brain areas like the perirhinal (PERI), entorhinal (ENT), and temporal associative cortices, as well as the prefrontal cortex (Aggleton and Brown, 1999). Moreover, ENT axons are the most significant cortical input to the hippocampus (Warburton and Brown, 2010). We therefore decided to analyze whether the functional activity of these areas is affected in *Lis1/sLis1* mutant mice. To this end, cellular activity in the PERI, ECT, and ENT





cortices was also analyzed through cFOS immunohistochemistry (**Figure 6**). We observed an increment of cFOS+ cells in PERI and ENT in *Lis1/sLis1* mutant mice compared to the controls ($n = 4$; **Figures 6A–D** average number of cFOS+ cells in the perirhinal area \pm SE: controls, 108 ± 8 , $n = 4$, *Lis1/sLis1*, 154 ± 11 , $n = 4$; $p < 0.01$, Student's *t*-test; average number of cFOS+ cells in the entorhinal area \pm SE: controls, 91 ± 10 , $n = 4$, *Lis1/sLis1*, 140 ± 11 , $n = 4$; $p < 0.01$, Student's *t*-test).

In contrast, no differences were observed when ECT was analyzed (**Figures 6A–D**; $n = 4$, average number of cFOS+ cells in the ectorhinal area \pm SE: controls, 129 ± 11 ; *Lis1/sLis1*, 148 ± 6).

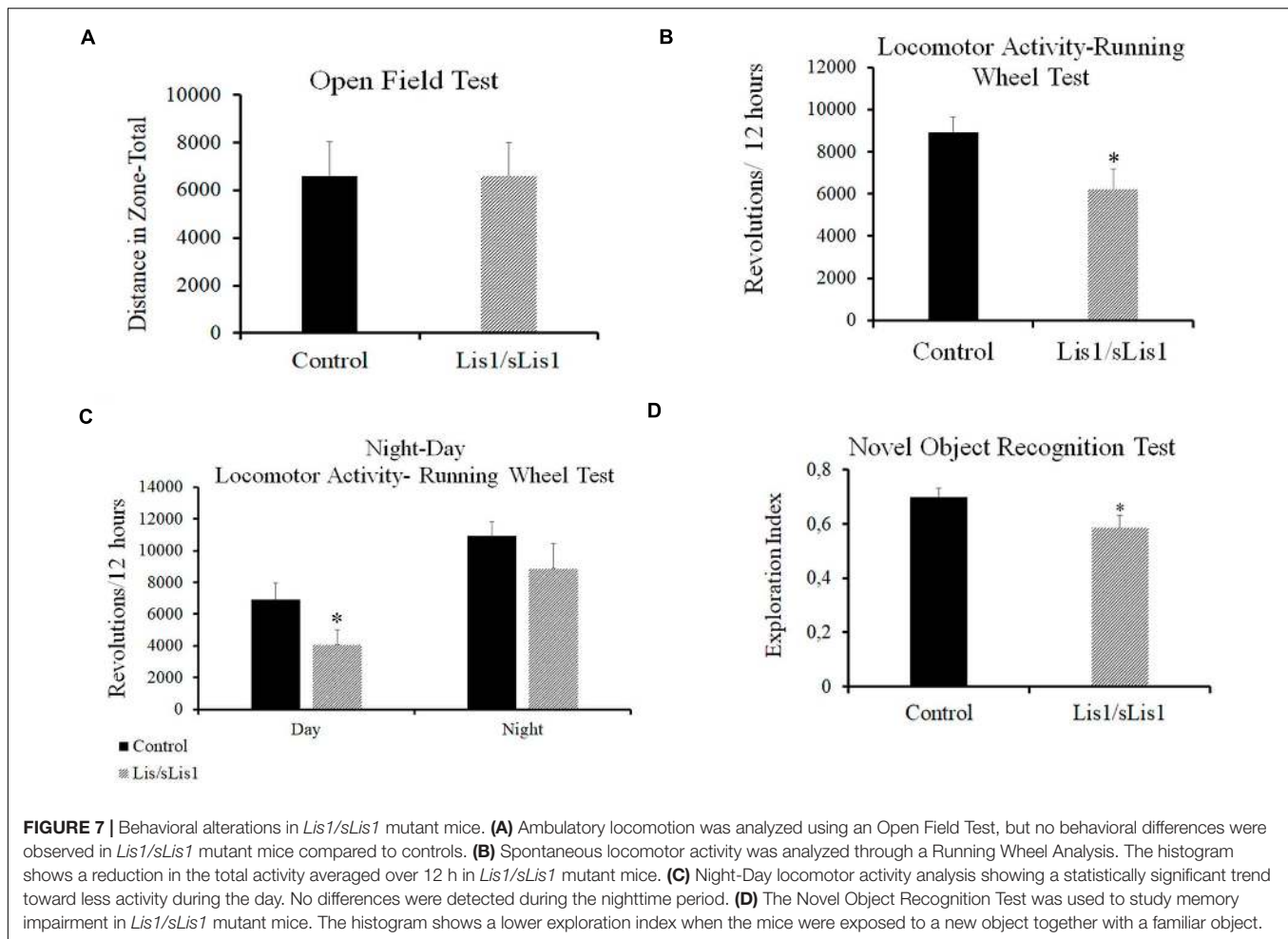
Our results show a significant increase in the number of cells expressing the cFOS protein in certain areas of the *Lis1/sLis1* brain, which mimic those described in postmortem brains of SZ patients and schizophrenia-like phenotype mouse models (Dragunow and Faull, 1990; Abdel-Naby Sayed et al., 2001; Keilhoff et al., 2004).

Behavioral Alterations in *Lis1/sLis1* Mutant Mice

To analyze the functional effects of the observed cortical alterations, behavioral analyses were performed. Firstly, ambulatory locomotor activity was assessed through the Open Field Test. The total distance traveled by the *Lis1/sLis1* group was

not significantly different to the control group ($n = 12$ for each group; **Figure 7A**).

Sleep disorders and circadian rhythm alterations have been described in SZ patients (Wulff et al., 2011). Furthermore, studies in animal models have suggested that schizophrenia-like phenotype mouse models display circadian rhythm disruption (Tam et al., 2015). We therefore studied spontaneous locomotor activity by means of a Running Wheel Analysis over a 72-h period. This test analyzes both circadian rhythms and locomotor activity. We observed decreased voluntary activity in the *Lis1/sLis1* mutant mice compared to the controls. Mutant mice made significantly fewer rotations than controls when considering a 12 h average (**Figure 7B**; $n = 12$ for each group, average number of revolutions/12 h \pm SE: controls, 8909 ± 737 , $n = 12$, *Lis1/sLis1*, 6213 ± 949 ; $p < 0.05$, Student's *t*-test). We then wanted to study whether these differences were due to a general decrease in activity and if there were differences between daytime and nighttime activity. Firstly, we analyzed the rest-activity rhythms in the control and mutant mice, respectively. Our results showed that both the controls and mutants presented statistically significant differences between their night vs. day activity ($n = 12$; $p < 0.01$ for control mice and $p < 0.001$ for *Lis1/sLis1* mutant mice). As expected, there was an increase in spontaneous locomotor activity at night in both control and mutant mice (**Figure 7C**). Next, we analyzed potential



night-day locomotor activity differences between control and *Lis1/sLis1* mutant mice. We observed more rest time during the day of *Lis1/sLis1* mutant mice, differences were statistically significant when compared with control mice. On the other hand, no significant differences in spontaneous locomotor activity were observed during the night (Figure 7C; $n = 12$ for each group, average number of revolutions/12 h \pm SE. Day: controls, 6901 ± 1041 , *Lis1/sLis1*, 4066 ± 923 ; $p < 0.05$, Student's *t*-test; Night: controls, 10918 ± 887 , *Lis1/sLis1* 8866 ± 1583).

Recognition memory deficits in SZ are an important cognitive symptom suffered by patients (Green et al., 2000; Tam et al., 2015). Memory deficits have also been reported in genetic SZ mouse models such as *Nrg1*-deficient mice (Harrison and Owen, 2003; Duffy et al., 2010; Pei et al., 2014) and in pharmacological, lesion and developmental SZ rodent models (Lyon et al., 2012). As our previous histological results showed that *Lis1/sLis1* mutant mice display quantitative and qualitative alterations of cell expressing cFOS in the hippocampus and in perirhinal and entorhinal cortices, we studied the possible existence of memory affectation. To this end, we performed the Novel Object Recognition (NOR) task. Impaired recognition memory was seen in *Lis1/sLis1* mice in this task. Mutant mice showed a significantly lower exploration index regarding the novel object compared to

the overall exploration than wild-type mice (Figure 7D, $n = 12$ for each group; average of the exploration index \pm SE: controls, 0.7 ± 0.03 , $n = 12$, *Lis1/sLis1*, 0.58 ± 0.045 ; $p < 0.05$, Student's *t*-test). No differences were observed between *Lis1/sLis1* mutant mice and controls in the training session (data not shown).

DISCUSSION

GABAergic Signaling in *Lis1/sLis1* Mutant Mice

The role of the *Lis1* gene in the development and function of the GABAergic system has been studied using a hypomorphic mutation of the *Lis1* allele (*Lis1/sLis1*) (Cahana et al., 2001, 2003), where the first coding exon from the *Lis1* gene has been deleted. Homozygous mice are not viable, but the study of heterozygous mice has revealed an abnormal neuronal morphology, cortical dysplasia, and enhanced excitability. Previous studies in our laboratory have demonstrated structural differences in cortical development (Cahana et al., 2001, 2003), the functional properties of cortical circuits (Valdés-Sánchez et al., 2007) and the basal forebrain, and alterations in septohippocampal projections in *Lis1/sLis1* mutant mice (Garcia-Lopez et al., 2015). In addition

to cortical morphology defects, we have linked variation in *LIS1* expression to human psychosis (Tabarés-Seisdedos et al., 2008). Here, we have examined *Lis1/sLis1* mutant mice to study how the altered *Lis1* gene expression might cause deficits associated with the pathophysiology of SZ.

We found that deleting the first coding exon of the *Lis1* gene affects the GABAergic cortical circuits. Many studies have related dysfunction of the GABAergic system with several cognitive impairments in SZ (Lewis et al., 2012; Sullivan and O'Donnell, 2012). Postmortem studies have shown decreased levels of mRNA from GABAergic markers, including glutamate decarboxylase 1 (GAD67) and parvalbumin (PV), with reduced PV expression being one of the most widely replicated findings in SZ (Akbarian et al., 1995; Guidotti et al., 2000; Fung et al., 2010; Gilabert-Juan et al., 2012; Glausier et al., 2014; Hoftman et al., 2015). Specifically, the mPFC and hippocampus seem to be the two main brain regions that present an altered GABAergic system in SZ (Heckers and Konradi, 2002; Heckers et al., 2002; Reynolds et al., 2004). Indeed, a significant reduction in the density of GABAergic inhibitory interneurons in the hippocampal region of schizophrenic brains has been previously reported (Benes and Berretta, 2001; Heckers and Konradi, 2002; Heckers et al., 2002). Moreover, studies of the hippocampus in SZ patients have reported a decreased population of the parvalbumin GABAergic interneurons (Zhang and Reynolds, 2002). In line with findings from studies in humans, deficits in GABAergic interneurons have been consistently described in different animal models of SZ (Jentsch and Roth, 1999; Thomsen et al., 2010; Ozdemir et al., 2012; Li J.-T. et al., 2016; Hauser et al., 2017). Our results demonstrate that the *Lis1/sLis1* hippocampus also shows a reduction in the number of cells expressing GAD67, in agreement with the observations made in other schizophrenia-like animal models. Interestingly, we observed a more severe reduction in CA3, which is innervated by ENT axons, either directly via the perforant path or indirectly from the dentate gyrus via the mossy fibers, the region with the most internal connectivity being in the hippocampal area (Amaral and Witter, 1989; Cherubini and Miles, 2015).

Several studies have also observed changes in other GABAergic interneuron subtypes, including CB, neuropeptide Y, somatostatin, vasoactive intestinal peptide, and cholecystokinin in the prefrontal cortex of SZ patients (Hashimoto et al., 2003, 2008; Fung et al., 2010, 2014). Furthermore, the anterior cingulate cortex (ACA) region of the brain presents an abnormal structure and function in SZ patients (Paus, 2001; Ridderinkhof et al., 2004; Rushworth et al., 2007). The ACA is a part of the mPFC that is involved in functions as diverse as associative learning, fear conditioning, and cognitive processes like attention. The functional heterogeneity of the ACA might explain the diversity of symptoms observed when this region is affected. These symptoms include apathy, inattention, dysregulation of autonomic function, and emotional instability, which are present in SZ (Picard and Strick, 1996; Andreasen et al., 1998; Vogt, 2005; Pietersen et al., 2007). To characterize our animal model with regard to the GABAergic system, we used GAD67, PV, and CR as protein markers. We found reduced numbers of GAD67+ interneurons in the ACA of *Lis1/sLis1* mutant mice compared to

control animals. In addition, we detected a selective reduction in the density in the PV- and CR-expressing sub-population of GABAergic interneurons in the ACA. These findings are also consistent with previous studies using genetic models of SZ that reported decreased densities of PV-positive interneurons (Flames et al., 2004; Fisahn et al., 2009; Fazzari et al., 2010; Ting et al., 2011). Our mouse model, however, did not present a decrease in the number of PV+ interneurons in the hippocampus as has been widely observed in postmortem studies in humans and schizophrenia-like mouse models.

Other studies reported that lower PV levels or fewer excitatory synapses on PV+ neurons were associated with SZ sufferers, where no loss of PV neurons was observed in postmortem analysis (Chung et al., 2016). That work reported a 12% reduction in VGLUT1 puncta on PV+ cell bodies in SZ subjects, demonstrating fewer synaptic glutamatergic structures on PV+ neurons. Similar results have been reported for mutant mouse models with a schizophrenia-like phenotype (Fazzari et al., 2010; Del Pino et al., 2013). The decreased excitatory input into PV+ cells in the hippocampus that we observed may be altering the excitatory-inhibitory balance in the hippocampus of *Lis1/sLis1* mutant mice.

Our results therefore suggest that *Lis1* gene dysfunction might be responsible for the anomalies observed in the GABAergic system, which are also described in the pathophysiology of SZ.

Genetic studies have shown several susceptible genes in SZ pathogenesis, such as the neuregulin 1 (NRG1) and ErbB4 receptors (Williams et al., 2003; Stefansson et al., 2004; Harrison and Weinberger, 2005; Del Pino et al., 2013; Wakuda et al., 2015). The *LIS1* gene (Reiner et al., 1995) encodes a non-catalytic subunit of platelet-activating factor acetylhydrolase-1b (PAFAH1b) (Hattori et al., 1994), a brain-specific enzyme that inactivates PAF. *LIS1* is one of the main genes related to Type I lissencephaly, a serious human brain malformation caused by the abnormal formation of the brain during development, the mildest mutations of which lead to a predisposition to several mental disorders (Dobyns et al., 1993; Reiner et al., 1995; Kato and Dobyns, 2003; Barkovich et al., 2005; Wynshaw-Boris, 2007; Tabarés-Seisdedos et al., 2008). At prenatal stages, when interneurons are being generated, the PAFAH1B complex (LIS complex) consists mostly of ALPHA1 and LIS1 dimers. Later in development, at perinatal stages, *Alpha1* levels decreased and *Alpha2* levels increased, to generate new complex forms. At stages E14.5–E17.5, *Lis1* is intensely expressed in both the VZ and SVZ layers in the pallium and in the subpallium (Escamez et al., 2012). sLIS protein is not capable of homodimerization and does not interact with LIS1 complex catalytic subunits (Cahana et al., 2001). It has been proposed that aberrant protein-protein interactions of sLIS1, could interfere in microtubules properties affecting cytoskeleton and causing defects in the cortical plate formation and in neuronal migration (Reiner, 2000). However, *Alpha1* transcript is expressed stronger in the subventricular zone (SVZ) of the ventral pallial region than in the dorsal and medial pallium suggesting a role in interneurons development. Interestingly, *Alpha1* expression is observed from early embryonic stages, with the highest peak at E14.5, until prenatal stages, and then it decreases. In *Lis1/sLis1* mutant mice

lower values of *Alpha1* expression were found during embryonic development and an abrupt decline at P0 (Escamez et al., 2012). The highest peak stage coincided with the expansion peak of the tangential migration of tangential interneurons in mice (Marín and Rubenstein, 2003). The decrease of *Alpha1* gene expression together with *Lis1* gene mutation in *Lis1/sLis1* mutant mice could explain why interneurons are specially affected in *Lis1/sLis1* mutant mice. The defective protein-protein interaction of sLIS1 protein together with the reduced expression of ALPHA1, described above, could be the biochemical basis of the phenotype observed in *Lis1/sLis1* mutant mice and could explain defects in interneuron development.

In addition, while mutation in other gene regions of *LIS1* have been associated to evident structural brain alterations described as lissencephalic spectrum disorders and associated to cerebral palsy and epilepsy (Pilz et al., 1998; Cardoso et al., 2002), deletion of the first coding exon shows a mild phenotype compatible with the histopathological data in SZ (Dobyns et al., 1993; Tabarés-Seisdedos et al., 2008).

It is well known that until late adolescence, the functional maturation of the GABAergic interneurons is not achieved, and it has been hypothesized that SZ could be associated with an alteration in the development of interneurons (Di Cristo, 2007; Hoftman and Lewis, 2011; Gonzalez-Burgos et al., 2015). Recently, it has been proposed that the maturation of all PV+ neurons in mouse PFC is rapid and almost completed by onset of puberty (Bitzenhofer et al., 2020). Thus, PV+ neuron maturation may contribute to the emergence of cognitive function primarily during prepubertal development.

Cells Expressing cFOS in *Lis1/sLis1* Mutant Mice

For the last three decades, the cFOS protein has been used as a marker for transcriptional activity (Sagar et al., 1988), and cFOS immunohistochemistry allows the labeling of stimulated neurons. Previous studies using pharmacological mouse models for SZ have shown that phencyclidine (PCP) and related drugs increased cFOS expression levels in the mPFC and hippocampus (Näkki et al., 1996; Sato et al., 1997; Kargieman et al., 2007; Santana et al., 2011; Castañé et al., 2015; Calovi et al., 2020). Moreover, it has been reported that cFOS expression induced by PCP occurs in GAD67+ interneurons, specifically in the hippocampus, RSC, amygdala, and somatosensory cortex (Santana et al., 2011). Interestingly, cFOS induced by PCP in PV+ interneurons was detected in cortical areas such as the ventrolateral orbitofrontal cortex, RSC, and CA1, whereas cFOS induction in the motor cortex co-labeled with CB+ interneurons (Hervig et al., 2016). Thus, in this study we used cFOS immunohistochemistry to compare the activation degree of distinct brain regions in *Lis1/sLis1* mutant mice. As we observed an important alteration in the GABAergic system that could have been affecting the excitation-inhibition balance in several cortical areas in our mutant mice, we decided to assess the neuronal activation in the cortex. As expected, we observed increased cFOS positive cells in *Lis1/sLis1* mutant mice in the same areas where the number

of interneurons was decreased. In fact, a significant increase in cFOS was found in the hippocampus, mPFC, ENT, and PERI compared to the control mice. These results suggest that *Lis1/sLis1* neuronal dysplasia originates an imbalance in neuronal activity in *Lis1/sLis1* mutant mice, as has been reported in other SZ mouse models. The reduction in interneurons (less GAD67-positive neurons) may underlay the hyperactivity of glutamatergic (pyramidal) neurons (cFOS overexpression). The ectopic activation and expression of cFOS has been proposed as a key factor responsible for the symptoms in SZ when PCP-related drugs are administered (Sharp et al., 1991; Post, 1992; Moghaddam et al., 1997). Indeed, increased cFOS expression has been suggested to play a role in negative symptoms observed in schizophrenia-like behavior (Abdel-Naby Sayed et al., 2001). On the other hand, some alleles of the *FOS* gene have been found to play a protective role, whereas others seem to be a risk factor for SZ, which has been connected to schizophrenia-related alterations in synaptic plasticity (Boyajyan et al., 2016). Interestingly, we observed higher cFOS activity in the DGsp of control mice, consistent with previous studies showing suprapyramidal-infrapyramidal gradients of neuronal activity in DG, which is not as functionally homogeneous as previously considered. Several observations have proposed that suprapyramidal (DGsp) and infrapyramidal (DGip) blades show different granule cell morphology and have asymmetric activity. Indeed, neurogenetic differences along the longitudinal (dorsal to ventral) and transverse (suprapyramidal and infrapyramidal) axes of the DG have been described (Jinno, 2011).

After a stimulus, DGsp is more quickly activated by entorhinal projections than DGip. The activation of DGsp is thought to occur together with inhibition to allow the DGsp to exceed DGip in response to the stimulation (Canning and Leung, 1997). Different levels of cFOS activity during spatial task performance have been described, with DGsp being more active during the task and DGip responding later (Scharfman et al., 2002; Chawla et al., 2005; Ramirez-Amaya et al., 2006; Marrone et al., 2012; Satvat et al., 2012; Gallitano et al., 2016). Interestingly, we found that the number of cFOS-positive cells in the infrapyramidal blade of the DG was greater in *Lis1/sLis1* mice compared to control mice, making the activity levels equivalent in both the DGsp and DGip of *Lis1/sLis1* mutants. Indeed, we found that whereas granule cells in the DGsp and DGip show different gradients of activation in control mice, these gradients are not present in the DG of *Lis1/sLis1* mutant mice, suggesting that there may be some dysfunction in terms of memory-related processes. Schmidt et al. (2012) described important anatomical and functional differences between DGsp and DGip blades that may be involved in pattern separation, and which are necessary to recognize small differences between similar stimuli and develop episodic memories (Schmidt et al., 2012).

These results suggest that the alteration of cFOS expression in DG granule cells disrupts activity patterns in DG circuits and, subsequently, causes cognitive alterations due to blade-dependent differences in excitability. This functional difference is not dependent on interneuron decreases, since these cells

are scarce in DG. The *Lis1/sLis1* mouse shows spontaneous disruption of the normal DG activity pattern, probably as a consequence of altered connectivity, which may represent a new model for studying the functional significance of differences in inter-blade activity in episodic memory development by using specific experimental paradigms.

A deviation of VGLUT1 fibers in the GCL of *Lis1/sLis1* mutant mice was described in a previous study (Wang and Baraban, 2008). These authors suggest that these fibers could target either different compartments of postsynaptic cells or completely inappropriate postsynaptic targets. The increase in excitation of regions of the DG in *Lis1/sLis1* mutants could be in line with this alteration of VGLUT1 fibers.

Behavioral Alteration in *Lis1/sLis1* Mutant Mice

The GABAergic system alteration and the increased cFOS-positive cells in specific cortical areas suggested that we should investigate the behavior of our mouse model. Sleep and circadian rhythm alterations have been observed in SZ patients, with a prevalence of up to 80% (Cohrs, 2008; Wulff et al., 2011). Such disruptions are severe and consist of advanced/delayed sleep phases or fragmented/excessive sleep periods (Wulff et al., 2011; Chan et al., 2017; Cosgrave et al., 2018). Interestingly, there are reports of a link between sleep alterations and psychotic symptoms, with increased risk of suffering one psychotic symptom when sleep disruption occurs (Lee et al., 2012; Koyanagi and Stickley, 2015; Oh et al., 2016). Moreover, sleep and circadian rhythm irregularities have also been described in genetic mouse models of SZ (Jeans et al., 2007; Yang et al., 2009; Oliver et al., 2012; Bhardwaj et al., 2015; Tam et al., 2015). We observed an alteration of the voluntary activity in the *Lis1/sLis1* mutant mice compared to the controls. The mutant animals made significantly fewer rotations than the controls. These differences were statistically significant in daytime periods, during which *Lis1/sLis1* mutant mice had longer rest periods than control mice. Other mouse models of SZ displayed a reduced amplitude of rest-activity or fragmented rhythms under constant dark conditions (Piggins, 2003; Oliver and Davies, 2009).

Our study also demonstrated that *Lis1/sLis1* mice demonstrate impaired recognition memory in the NOR task. This test evaluates the perirhinal cortex-dependent, familiarity-based recognition memory. Since PERI and DG are the most important brain regions with regard to object recognition memory (Zola-Morgan et al., 1991; Winters, 2005), these results agree with that expected according to the structural and functional alterations observed in these regions. Damaged recognition memory is one of the main cognitive symptoms of SZ patients (Danion et al., 1999; Thoma et al., 2006; Tam et al., 2015). In addition, this impairment has also been reported in SZ mouse models in both genetic (Duffy et al., 2010; Pei et al., 2014) and pharmacological (Li C. et al., 2016) models.

In summary, we observed that the *Lis1/sLis1* mutant mice presented two important behavioral alterations frequently linked to a schizophrenia-like phenotype: altered spontaneous

locomotor activity during daytime periods; and impaired recognition memory.

CONCLUSION

Our mouse model could represent schizophrenia in two main aspects: the pathological and the symptomatic. *LIS1* is a gene previously related to risk of developing schizophrenia in humans and it could be one of the physiopathological processes underlying schizophrenia. Moreover, we have observed a group of symptoms widely observed in other models of schizophrenia and in human patients. One of the strong points of the genetic models for neurological diseases is that they are able to accurately represent the genetic basis of human disorders (Loring et al., 1996; Lipska and Weinberger, 2000). Here, we used a genetic model in which the gene *Lis1* has a mutation similar to the mutations described in humans (Cahana et al., 2001; Reiner et al., 2002). Unfortunately, a single analysis to test the fidelity of a schizophrenia mouse model is missing. Moreover, no animal can completely reproduce the symptoms of schizophrenia as they are present in humans. In this situation animal models for schizophrenia should reproduce a group of behavioral and biological phenomena relevant to schizophrenia (Lipska and Weinberger, 2000).

Schizophrenia has an important heritable component with a complex genetic basis and a strong neurodevelopmental component (Kendler et al., 1996). Based on these facts, it is important to focus the problem from perspectives other than the pharmacological one. *Lis1/sLis1* mice could be considered a genetic model affecting neurodevelopment. In our work we have shown that *Lis1/sLis1* mice exhibit histological, brain activation, and behavioral abnormalities reminiscent of schizophrenia: reduction in the number of interneurons in ACA, disbalance in cellular activation assessed by c-fos expression and the alteration of behavioral tests related to these cellular deficiencies. Our results demonstrate that *Lis1/sLis1* mutant mice exhibit an important developmentally originated alteration of the inhibitory system in cortical areas engaged in associative learning, memory, and attention. We observed that the mutation of the *Lis1* gene in early development might not only alter the number of cells positive of GAD67, CR, and PV but may also modify synapse formation decreasing VGLUT1+ terminals contacting PV+ interneurons. These alterations in the developing brain may produce a dysfunction in the activation balance of the cerebral cortex, increasing the cFOS activity, affecting the developmental process and producing long-term behavioral alterations. Specific alterations observed in DG blade activation represent a new model for understanding the functional relevance of this heterogeneous pattern.

Since the phenotype described in this animal resembles characteristics and symptoms described in SZ patients and in validated animal models for SZ, we conclude that cortical dysplasia when not severe, as is the case in *Lis1/sLis1* mutant mice, represents a pathogenetic mechanism of SZ. Moreover, alteration of the *Lis1* gene expression could be related to the risk of developing SZ.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The animal study was reviewed and approved by the Ethics Committee in Animal Experimentation of the Miguel Hernandez University (2016/VSC/PEA/00190 and 2017/VSC/PEA/00211).

AUTHOR CONTRIBUTIONS

RG-L and AP: conceptualization, investigation, and writing – original draft preparation. AE: methodology and resources. EG-B: supervision and review. SM: conceptualization, supervision, writing – review and editing, and funding acquisition. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fcell.2021.693919/full#supplementary-material>

Supplementary Figure 1 | Genotyping by PCR of *sLis1* (by using one set of primers). 0.1300 kb is the *sLis1* band. Electrophoresis gel shows the molecular weight pattern, a positive control, a negative control and a representative example of a heterozygous mouse.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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