Research paper

PlexinA1-deficient mice exhibit decreased cell density and augmented oxidative stress in parvalbumin-expressing interneurons in the medial prefrontal cortex

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ABSTRACT

PlexinA1 (PlxnA1) is a transmembrane receptor for semaphorins (Semas), a large family of axonal guidance cues vital during neural development. PlxnA1 is expressed in embryonic interneurons, and PlxnA1 deletion in mice leads to less interneurons in the developing cortex. In addition, PlxnA1 has been identified as a schizophrenia susceptibility gene. In our previous study, PlxnA1 knockout (KO) mice under a BALB/cAJ genetic background exhibited significantly increased self-grooming and reduced prepulse inhibition, a reliable phenotype for investigating the neurobiology of schizophrenia. However, the mechanism underlying the abnormal behavior of PlxnA1 KO mice remains unclear. We first confirmed PlxnA1 mRNA expression in parvalbumin-expressing interneurons (PV cells) in the medial prefrontal cortex (mPFC) of adult mice. Immunohistochemical analysis (IHC) showed significantly decreased densities of both GABAergic neurons and PV cells in the mPFC of PlxnA1 KO mice compared with wild type mice (WT). PV cells were found to express molecule interacting with CasL 1 (MICAL1), an effector involved in Sema-Plxn signaling for axon guidance, suggesting MICAL1 and PlxnA1 co-expression in PV cells. Furthermore, IHC analysis of 8-oxo-dG, an oxidative stress marker, revealed significantly increased oxidative stress in PlxnA1-deficient PV cells compared with WT. Thus, increased oxidative stress and decreased PV cell density in the mPFC may determine the onset of PlxnA1 KO mice’s abnormal behavior. Accordingly, deficient PlxnA1-mediated signaling may increase oxidative stress in PV cells, thereby disrupting PV-cell network functions in the mPFC and causing abnormal behavior related to neuropsychiatric diseases.

1. Introduction

PlexinA1 (PlxnA1) is a neuronal receptor for the semaphorin (Sema) family of axon guidance molecules (Kong et al., 2016, Winberg et al., 1998). PlxnA1 is known for its expression on the surface of axonal growth cones where it acts as a guidance receptor for developing axons toward their targets (Delloye-Bourgeois et al., 2015). The direction of axonal elongation is determined by the action of axon guidance molecules, including Semas (Kong et al., 2016). The receptor-ligand pairing of Plxn and Sema along with neuropilins (Nrp) regulate a wide range of cellular processes in developmental and adult physiology (Junqueira Alves et al., 2019). In addition to their axon guidance properties, PlxnA1

Abbreviations: PlxnA1, PlexinA1; KO, knockout; mPFC, medial prefrontal cortex; WT, wild type mice; MICAL1, molecule interacting with Casl. 1; Sema, semaphorin; PNNs, perineuronal nets; PV cells, parvalbumin-expressing interneurons; SST cells, somatostatin-positive cells; CR cells, calretinin-positive cells; PND, postnatal day; 8-oxo-dG, 8 hydroxyguanine.

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is involved in cell migration and synaptic development (Andres et al., 2016; Waihney and Cheng, 2006). Sema3A/PlxnA1 signal regulates the activity dependent patterning of dendritic arbors in hippocampal neurons through Farp1 (Cheadle and Biederer, 2014). PlxnA1 and PlxnA3 mediate Sem5A and Sema5B signal which constrain neurites from many retinal neuron subtypes within the inner plexiform layer (Mat-suoka et al., 2011). PlxnA1 is also involved in Semd6-mediated axon pruning which eliminates ipsilateral projections of corticospinal neurons in the spinal cord through activity-induced Bax-caspase signaling pathway (Gu et al., 2020). In all these processes, the molecule interact- ing with Gskl (MCAL) family proteins, which are flavoprotein monoxygenase/hydroxylase enzymes, may play key roles by dis-assembling actin filaments by directly oxidizing actin’s methionine residues during Plxn-mediated Semas signaling (Alto and Terman, 2018; Lee et al., 2013; Hung et al., 2013; Hung et al., 2010; Terman et al., 2002). In the embryo, PlxnA1 is expressed in cortical interneurons and plays an important role in forebrain development (Andres et al., 2016). PlxnA1 is also expressed in interneurons in the adult mouse cortex, surrounded by perineuronal nets (PNNs) containing Sema3A, a class 3 Sema ligand (Yi et al., 2013). In a previous study, we showed PlxnA1 mRNA expression in GABAergic interneurons of the adult mouse prefrontal cortex and hippocampus, indicating that PlxnA1 may also act in these brain areas throughout adulthood (Jahan et al., 2020).

The prefrontal cortex (PFC) and hippocampus assume a central role in various behavioral and intellectual processes (Sigurdsson and Duvarcir, 2015). GABAergic neurons essentially maintain the neural hardware of these regions (Sun et al., 2020). In the central nervous system (CNS), GABAergic neurons comprise a heterogeneous group of cells, including ~20% of the total neuronal populations (Xu et al., 2010; Markram et al., 2004; Aika et al., 1994; Halasy and Somogyi, 1993). In the rodent cerebral cortex, parvalbumin-expressing interneurons (PV cells) are the largest population of GABAergic neurons, accounting for about 40%, whereas somatostatin-positive cells (SST cells) constitute around 30% of cortical GABAergic neurons and calretinin-positive cells (CR cells) the remaining 25% (Hladnik et al., 2014). Inhibitory GABAergic interneurons in the adult CNS regulate the activity of neural circuits by regulating the inhibitory control of target neurons through ionotropic GABA receptors (Ghosal et al., 2017). The harmony between excitatory and inhibitory signals is required for normal memory and emotional behavior and is disrupted in many psychiatric disorders (Nelson and Valakh, 2015; Ben-Ari, 2014; Lewis et al., 2012). Moreover, both excitatory and inhibitory neurons are guided during migration from the birth places to the embryonic cortices by various chemo-attractive and repulsive cues (Marín et al., 2001; Mêtin et al., 2006; O’Leary and Bongrass, 2006).

Neuropsychiatric disorders have profoundly intricate and diverse etiologies. Genetic studies have shown that genetic alterations including copy number variations, single nucleotide polymorphism, and de novo gene mutations in combination with environmental stress greatly contribute to the underlying pathogenesis (Bray and O’Donovan, 2019). Recently, Plxn has attracted attention as a potential cause of nervous system disorders like schizophrenia (Gilabert-Juan et al., 2015; Mah et al., 2006), Alzheimer’s disease (Jun et al., 2014), Parkinson’s disease (Schulte et al., 2013), epileptic encephalopathy (Olive et al., 2016), or developmental encephalopathy (Park et al., 2017), among others. One study revealed a missense variant (NM_022242, c.4201 G > A, p.1401 M > L) of the PlxnA1 gene in adult humans with schizophrenia (Park et al., 2014). Case-control cohort analysis identified PlxnA1 as a de novo schizophrenia-related gene and found an association of disruptive (missense and frameshift) mutations in PlxnA1 and schizophrenia in a Swedish population (Purcell et al., 2014). In adult PlxnA2-/- mice, the dentate granule cell layer is severely malformed, neurogenesis is compromised, and behavioral studies revealed deficits in associative learning, sociability, and sensorimotor gating traits; all schizophrenia-like behaviors (Zhao et al., 2018). Moreover, deregulation of Sema3A and PlxnA1 transcript levels was reported in the PFC of schizophrenic patients (Gilabert-Juan et al., 2015).

We previously performed behavioral experiments on adult PlxnA1 knockout (KO) mice and found that PlxnA1 KO mice showed impaired postnatal development and abnormal behaviors like excessive stereotypic hyper-grooming, reduced anxiety-like behavior, and impaired prepulse inhibition, recapitulating some aspects of schizophrenia and other neurodevelopmental disorders (Jahan et al., 2020). PlxnA1 KO mice develop enhanced self-grooming and deficit of prepulse inhibition at 6 months of age. Those abnormal behaviors do not significantly appear at younger age like 2 months of age (Jahan et al., 2020). The age-dependency of the abnormal behaviors do not support that decrease of interneurons in developing cortex at embryonic day 18.5 (E18.5) in PlxnA1 KO mice (Andres et al., 2016) is a direct cause of the behavioral deficits. It remains unclear if the decrease of interneurons in PlxnA1 KO mice persists even in postnatal cortex where laminar allocation, apoptosis and maturation of interneurons occur. It is rather possible that long term and progressive alterations in postnatal development, maturation and function of interneurons may result in the behavioral deficits.

PV cells become enwrapped by perineuronal nets (PNNs) on the maturation of PV cells during postnatal period (Giamanco et al., 2010). It is indicated that Sema3A, a constituent of PNNs contributes to PNN-mediated restriction of ocular dominance plasticity at the closure of the critical period in the visual cortex (Boggio et al., 2019). PlxnA1 and PlxnA4 exist on the membrane of PV cells (Vo et al., 2013), although their roles remain unknown. There are several hypotheses on the mechanism how Sema3A in PNNs exerts influence on neurons, plasticity and/or connectivity through the interaction with its receptors on the membrane of postsynaptic neurons or presynaptic terminals (Carulli and Verhaagen, 2021).

Sema3A is crucially involved in migration of interneurons from the medial ganglionic eminence (MGE) to developing cortex (Andres et al., 2017). Loss of PlxnA1 causes reduced proliferation of interneurons thus leading to fewer interneurons in developing cortex, as well as interneurons and projection cells at E14.5 and E18.5 (Andres et al., 2016). PlxnA1 may be redundant in the migration of interneurons from MGE to cortex (Andres et al., 2016). It can be anticipated that cortical interneurons exhibit the decrease in cell density during postnatal period by the influence of decreased interneurons in embryonic cortices of PlxnA1 KO mice. However, interneuron density in PlxnA1-deficient cortex may come near WT during postnatal period by receiving the influences such as migration lasting even after E18.5, balancing of interneuron number by postnatal apoptosis (Duan et al., 2020), and the genetic background of mice. Thus, to study a possible role of PlxnA1-deficiency in the onset of these abnormal mouse behaviors related to human psychiatric disorders, the present study examined the density of GABAergic interneurons, and PV, SST, and CR cells in the medial prefrontal cortex (mPFC), dorso-lateral frontal cortex (DLFC), and hippocampus of PlxnA1 KO mice. Moreover, the study further quantified the oxidative stress in PV cells in the mPFC of both WT and PlxnA1 KO mice to investigate the reasons underlying the lower density of PV cells in the mPFC and the abnormal behavior of PlxnA1 KO mice.

2. Materials and methods

2.1. Generation of PlxnA1 KO mice

PlxnA1 KO mice were produced by gene targeting with E14.1 embryonic stem (ES) cells (Takegahara et al., 2006). Briefly, a gene targeting vector was designed to replace the genomic region containing the initiation codon and the Sema domain-coding sequence by a neomycin resistance gene; then, it was transfected into E14.1 ES cells by electro- poration. G418- and ganciclovir-resistant clones were screened by po-sitive selection gene; then, it was transfected into E14.1 ES cells by elec-
mice and backcrossed with BALB/cAJ mice (CLEA Japan, Inc., Tokyo, Japan) for 10 generations. The resultant heterozygous mice were then bred to produce homozygous KO mice and WT littermates were used as controls. Both WT and PlxnA1 KO mice were subjected to the behavioral tests at 6 months of age (Jahan et al., 2020) and sacrificed for immunohistochemical studies on postnatal day (PND) 190.

2.2. Animals

Mice were reared in the animal center in the Faculty of Pharmacy of Meijo University. WT and PlxnA1 KO mice were housed in littermate groups under a controlled environment (23.1 °C, humidity 50.5%, illuminance 300 lux, 12 h:12 h light/dark cycle) with water and food ad libitum (MM-3, Sanko Labo Service Corporation, INC., Tokyo, Japan). Pups were weaned on PND24, housed in groups of five per cage. Mouse care and the experimental protocols were executed according to the guidelines of the Physiological Society of Japan and the guidelines on animal experimentation of Meijo University. The Animal Ethics Review Committee of Meijo University approved the experimental protocol (authorization number: 2020PE4).

2.3. Genotype analysis

Genotyping of both WT and PlxnA1 KO mice was confirmed by PCR using mouse tail DNA as template and a previously reported PlxnA1 gene-specific primer set (Ito et al., 2014; Takegahara et al., 2006).

2.4. In situ hybridization of PlxnA1

To analyze PlxnA1 mRNA co-expression in PV cells, in situ hybridization was performed according to the manufacturer’s protocol using RNAscope Probes (Advanced Cell Diagnostics (ACD), CA, USA) targeting the 1091–1984 base pair region of the mouse PlxnA1 gene (Accession No: NM_008881.2). RNAscope Target Probe Mm-PlexinA1 (cat no. 481388, ACD, USA) was used to examine PlxnA1 expression. RNAscope Positive Control Probe Mm-Ppib (cat No.313911, ACD) and RNAscope Negative Control Probe-DapB (cat no.310043, ACD) were used as a positive and negative control to test optimal permeabilization and probe RNA quality, respectively. To confirm PlxnA1 mRNA localization in PV cells, immunohistochemistry was performed following the in situ hybridization. To this end, after soaking samples in PBS exchanged three times for 5 min, incubation was performed with protein block serum free (Dako, code X0909, CA, USA) for 1 h at room temperature (RT). An anti-PV (1:200, PV27, Swant, Switzerland) antibody was used as a primary antibody overnight at 4 °C. Alexa Fluor 488 donkey anti-rabbit (1:1000, A21206, Thermo Fisher Scientific, USA), Alexa Fluor 555 donkey anti-mouse (1:1000, A21370, Thermofisher Scientific, USA), and Alexa Fluor 488 donkey anti-rat (1:500, A21208, Thermofisher Scientific, USA) were used for secondary antibodies. Secondary antibodies were incubated with blocking buffer, protein block serum free (Dako) at RT for 1 h, and incubated overnight with primary antibodies at 4 °C. Primary antibodies used for the detection of target proteins during immunostaining were anti-GABA antibody (1:500, A2052, Sigma–Aldrich, MO, USA), anti-parvalbumin antibody (1:1000, MAB1572, Merck, CA, USA), anti-calretinin antibody (1:4000, MAB1568, Merck), and anti-somatostatin antibody (1:250, A20067, Immunostar, Wisconsin, USA). On the second day, sections were washed three times in PBS for 30 min and incubated with the following secondary antibodies diluted with blocking buffer. Secondary antibodies used as follows: Alexa Fluor 488 donkey anti-rabbit (1:1000, A21206, Thermofisher Scientific, USA), Alexa Fluor 555 donkey anti-mouse (1:1000, A21370, Thermofisher Scientific, USA), and Alexa Fluor 488 donkey anti-rat (1:500, A21208, Thermofisher Scientific, USA). Sections were washed in PBS three times for 15 min and finally covered with micro cover glasses (Matsunami Glass Industries Ltd.) using Fluoro-KEEPER Antifade Reagent, Non-Hardening Type with DAPI (cat no. 12745-74, Nacalai Tesque Inc.). The prepared slides were either imaged immediately or stored at 4 °C until imaging. 4 consecutive sections per mouse were used for the same primary antibody in the immunohistochemistry.

2.5. Tissue preparation

Mice were anesthetized with a mixture of sodium pentobarbital (40 mg/Kg) and medetomidine (0.3 mg/Kg) intraperitoneally after the behavioral tests (Jahan et al., 2020). Then, transcardiac perfusion with ice-cold PBS at pH 7.4 for 4 min 30 s and successive perfusion with 4% paraformaldehyde in PBS (pH 7.4) for 7 min 30 s (4.1 mL/min) with a peristaltic pump (BIORAD; Japan) were conducted from the left ventricle. After perfusion, the brains were dissected and post-fixed in the same fixative for 2 h at 4 °C. Then, the brain was sequentially immersed with 10% sucrose solution, followed by 20% and 30% sucrose solution in PBS for two consecutive days at 4 °C. Embedding of the fixed brain was performed in O.C.T. compound (Sakura Finetek Japan Co. Ltd., Tokyo, Japan) using liquid nitrogen.

2.6. Immunohistochemistry

Embedded brains were sectioned coronally at 20 μm thickness in –20 °C on a Leica CM 1860 UV (Leica Biosystems Nussloch GmbH, Nussloch, Germany) at the PFC level (ranging 2.58–2.96 mm from bregma) and hippocampus (ranging –1.58 to –2.06 mm from bregma) according to the mouse brain atlas (The Mouse Brain in Stereotaxic Coordinates, Keith B.J. Franklin & George Paxinos, 3rd edition). The sections were mounted on an adhesive glass slide (MAS-05, Matsunami Glass Industries Ltd.) dried for 1 h and processed for staining. At first, the cryostat sections were washed in PBS with 0.1% polyoxyethylene sorbitan monolaurate (cat no. 166–21213, Wako Pure chemical industries Ltd., Osaka, Japan) for 15 min. After three washes in PBS for 5 min, the sections were heated in antigen retrieval solution, 10% HistoVT One (cat no. 06380–05, Nacalai Tesque Inc.) solution for 20 min at 75 °C and left for 20 min at RT for cooling down. The slides were washed in PBS for 15 min and marked around the sections with a liquid-blocking pen (Liquid blocker, DAIDO SANGYO, Saitama, Japan). Sections were incubated with blocking buffer, protein block serum free (Dako) at RT for 1 h, and incubated overnight with primary antibodies at 4 °C. Primary antibodies used for the detection of target proteins during immunostaining were anti-GABA antibody (1:500, A2052, Sigma–Aldrich, MO, USA), anti-parvalbumin antibody (1:1000, MAB1572, Merck, CA, USA), anti-calretinin antibody (1:4000, MAB1568, Merck), and anti-somatostatin antibody (1:250, A20067, Immunostar, Wisconsin, USA). On the second day, sections were washed three times in PBS for 30 min and incubated with the following secondary antibodies diluted with blocking buffer. Secondary antibodies used as follows: Alexa Fluor 488 donkey anti-rabbit (1:1000, A21206, Thermo Fisher Scientific, USA), Alexa Fluor 555 donkey anti-mouse (1:1000, A21370, Thermofisher Scientific, USA), and Alexa Fluor 488 donkey anti-rat (1:500, A21208, Thermofisher Scientific, USA). Sections were washed in PBS three times for 15 min and finally covered with micro cover glasses (Matsunami Glass Industries Ltd.) using Fluoro-KEEPER Antifade Reagent, Non-Hardening Type with DAPI (cat no. 12745-74, Nacalai Tesque Inc.). The prepared slides were either imaged immediately or stored at 4 °C until imaging. 4 consecutive sections per mouse were used for the same primary antibody in the immunohistochemistry.

2.7. 8-oxo-dG immunofluorescence as marker of oxidative stress

Immunohistochemical studies were performed to assess oxidative stress of PV cells in the mPFC of PlxnA1 KO mice. WT (n = 6) and PlxnA1 KO mice (n = 5) were immunostained with anti- PV antibody (1:400, PV 27) and anti-8 hydroxyguanine (8-oxo-dG) antibody (1:400, 4354-MC-050, R&D, Trevigen, MD, USA) according to the above immunohistochemistry procedure. The secondary antibodies utilized were Alexa Fluor 555 donkey anti-rabbit (1:1000, A21370, Thermofisher Scientific) for the anti-PV antibody and Alexa Fluor 488 donkey anti-mouse (1:200, A21202, Thermofisher Scientific) for the anti-8-oxo-dG antibody. To observe MICAL1 expression in neurons, its co-expression with PlxnA1 mRNA, and co-localization with PV in PV cells, an anti-MICAL1 rabbit polyclonal antibody (1:200, Cat. No. 14818–1-AP, Proteintech, USA) was used for analysis.

2.8. Microscopy imaging

The samples were imaged with an All-in-One Fluorescence Microscope (BX-X710; Keyence) controlled with BX-X viewer version 1.3.1.1. and analyzed with BX-X Analyzer version 1.4.0.1. Images from brain sections (3 to 4 sections/mice) were obtained using a × 20 objective of the fluorescence microscope and merged with the analyzer software. Then, a 500 × 1000 μm² (441 × 883 pixels) area in the mPFC region covering the prelimbic cortex and medial orbital cortex (Fig. 1A) was...
selected according to the borders in the mouse brain atlas (The Mouse Brain in Stereotaxic Coordinates, Keith B.J. Franklin & George Paxinos, 3rd edition). In the DLFC, 600 × 1200 µm² (1059 × 529 pixels) area were selected for measurement (Fig. 1A). The whole hippocampus (right & left, Fig. 1B) was captured as above. Prior to image capture, the exposure time, gain, and offset were carefully set to ensure a strong signal but to avoid saturation. Identical capture conditions were used for all sections.

2.9. Quantification of neuron density and image analysis

Using the integrated cell counter plugin in ImageJ software, immunolabeled cells from various region of interest (ROIs) were counted (ImageJ 1.46r, NIH, USA, http://imagej.nih.gov/ij). All neuronal cell bodies visualized with the nuclear marker DAPI were manually marked and counted in 100 × view on ImageJ. For counting, cells with length of < 5 µm were excluded to avoid counting neurites and false signals. The density of immunolabeled cells was calculated for a single ROI in each

Fig. 1. PlxnA1 mRNA expression in PV cells of adult mice. In situ hybridization with a PlxnA1 mRNA-specific probe followed by immunofluorescence with an anti-PV antibody shows PlxnA1 mRNA expression (red) in the mPFC and DLFC (A), and in the cerebral cortex and hippocampus (B). In DLFC, 6 layers (each: 200 µm high) are arbitrarily assigned following previous report (Shen et al., 2008). The analysis reveals PlxnA1 mRNA expression (red) in PV cells (green) with 20 × view (C), PlxnA1 mRNA (D: red), PV cells (E: green), PlxnA1 mRNA in PV cells in merged view (F: arrows) in the mPFC of WT. Images of the mPFC with measured density of immunolabeled cells with anti-PV antibody are shown for WT (G) and PlxnA1 KO mice (H). Blue color: DAPI for nuclear stain (A, B). WT: wild type mice, PlxnA1 KO: PlxnA1-deficient mice. PrL: prelimbic cortex, MO: medial orbital cortex, AON: anterior olfactory nucleus, DG: dentate gurus, Scale bars: 1000 µm (A), 500 µm (B), 200 µm (C), 50 µm (D, E, F, G, H).
section, and the mean cell density per ROI (cells/mm$^2$) in each mouse was obtained to compare the density between PlxnA1 KO mice and WT controls. Two experimenters blind of genotypes independently performed cell counting to increase the accuracy. 8-oxo-dG fluorescence intensity was measured over each PV cells residing in a 400 x 400 μm$^2$ counting area set in the prelimbic cortex in mPFC. Briefly, using ImageJ’s freehand tool, in the red channel an outline was drawn around each cell positively immunolabeled for PV and then switched into green channel and measured the mean fluorescence intensity of 8-oxo-dG in each PV cell. All intensity measurements were performed on an 8-bit scale green image. We gained the average of the mean fluorescence intensity in 8 counting areas from 4 sections per mouse, and compared the average of the mean fluorescence intensity of 8-oxo-dG between WT and PlxnA1 KO mice (WT: n = 6, KO: n = 5).

2.10. Data analysis

Statistical analysis was conducted using GraphPad prism 8 (version 8.4.1(676), GraphPad software Inc., San Diego, CA, USA). Data were analyzed by the unpaired Student’s t-test and shown as mean ± SEM. All immunohistochemistry results are expressed as cells/mm$^2$. The level of significance was set at p < 0.05.

3. Theory/calculation

The present study explored the mechanism underlying the abnormal behavior of PlxnA1 KO mice. The first hypothesis was that GABAergic circuitry formation may be impaired by the developmental defects of embryonic interneurons in PlxnA1 KO mice (Andrews et al., 2016). The second hypothesis was that a subpopulation of GABAergic interneurons may be impaired by the long-term persistence of postnatal effects such as oxidative stress caused by PlxnA1-deficiency. Blockade of Semaphorin signaling may augment oxidative stress in neurons and glia (Xu et al., 2021; Li et al., 2019). Among GABAergic interneurons, PV cells especially vulnerable to oxidative stress are affected by chronic redox dysregulation (Cabungcal et al., 2013). In experiments to clarify the mechanism, we confirmed the PlxnA1 mRNA expression in PV cells in the mPFC and detected a significant reduction of PV cells in the mPFC of PlxnA1 KO mice. Since there was no significant decrease in PV cell density in the mPFC of PlxnA1 KO mice at 2 weeks of age, possibility of the developmental defects was considered lower. Thus, we focused on oxidative stress in PV cells in the mPFC. After confirming MICAL1 expression in PV cells in the mPFC, significantly increased DNA oxidation was detected in PV cells of the mPFC in PlxnA1 KO mice than in WT. Thus, we propose that PlxnA1-deficiency may cause the increased oxidative stress in PV cells during long-lasting postnatal life, subsequently the reduction of PV cell density in the mPFC, and finally the abnormal behavior.

4. Results

4.1. PlxnA1 mRNA expression in PV cells

Our previous study detected PlxnA1 mRNA expression in GABAergic neurons in the PFC and hippocampus of adult mice (Jahan et al., 2020). To examine PlxnA1 mRNA expression in PV cells, we performed in situ hybridization in WT. PlxnA1 mRNAs were scattered throughout all PFC layers, as well as all subfields of the hippocampal region (Fig. 1A, B). In addition, double labeling revealed expression of PlxnA1 mRNAs in PV cells in the mPFC (Fig. 1C-F), hippocampus, and cortex of adult mice (data not shown). 42% of PV cells (27 out of 64 PV cells) in mPFC expressed PlxnA1 mRNAs (Fig. 1C, F). 51% of GAD67-GFP positive interneurons (116 out of 227 cells) in mPFC expressed PlxnA1 mRNAs (Jahan et al., 2020; Tamamaki et al., 2003). The result indicates that PlxnA1 mRNAs are also expressed in other interneuron subtypes other than PV cells.

4.2. Reduction of GABA-positive cells in the mPFC of PlxnA1 KO mice

After detecting PlxnA1 mRNA expression in GABAergic interneurons, we examined the density of GABAergic interneurons and their subtypes in the PFC and hippocampus. Coronal sections containing the mPFC, DLFC, and hippocampus in WT and PlxnA1 KO brains at 6 months of age were immunostained for identification of GABAergic neurons (de Lima et al., 2007; Voigt et al., 2001). In the mPFC, there was a significant reduction (Fig. 2A–D; G; for each group: n = 5, t (8) = 2.92, p = 0.03, p < 0.05) of GABA-positive cells in PlxnA1 KO mice compared with WT littermates. In the DLFC, GABA-positive cell density did not significantly differ between PlxnA1 KO mice and WT (Fig. 2E, F; H; for each group: n = 5, t (8) = 0.29, p = 0.78, p > 0.05). In hippocampal regions, there was no significant difference in GABA-positive cell density between WT and PlxnA1 KO mice (Supplementary Fig. 1A, B, E; for each group: n = 5, t (8) = 0.99, p = 0.39, p > 0.05).

4.3. Decreased PV cells density in the mPFC of PlxnA1 KO mice

To identify the subpopulation of GABAergic interneurons affected by PlxnA1 deficiency, the cell density of PV, CR, and SST cells was measured by immunohistochemical analyses. At 6 months of age, PlxnA1 KO mice showed significantly decreased PV cell density in the mPFC compared to WT littermates (Fig. 3A, B, C, D, G; for each group: n = 6, t (10) = 2.60, p = 0.03, p < 0.05). However, at 2 weeks of age, PlxnA1 KO mice did not show any significant decrease in PV cell density in the mPFC compared with WT littermates (WT (n = 5); 142.0 ± 7.944 cells/mm$^2$, KO (n = 4); 131.5 ± 6.278 cells/mm$^2$, t (7) = 0.9942, p = 0.41, p > 0.05, Supplementary Fig. 2). In the DLFC and hippocampus, there was no significant difference in PV cell density between WT and PlxnA1 KO mice (DLFC: Fig. 3F, H; for each group: n = 5, t (8) = 1.66, p = 0.13, p > 0.05 and Supplementary Fig. 1C, D, F; for each group: n = 5, t (8) = 0.97, p = 0.34, p > 0.05 in hippocampus), respectively. In the DLFC, there were no significant differences of PV cell density between both genotypes in arbitrarily divided 3 layers (modified by Shen et al., 2008; Supplementary Fig. 3; for each group, n = 3, genotype: F (1, 2) = 0.6871, p = 0.4943, p > 0.05; layer: F (1.309, 2.617) = 7.063, p = 0.0885, p > 0.05). In the CA3 region of hippocampus, PlxnA1 KO mice showed significantly decreased PV cell density as compared with WT (Supplementary Fig. 4; for each group: n = 5, t (8) = 2.720, p = 0.0263, p < 0.05). In the CA1 and dentate gyrus (DG), there was no significant difference in PV cell density between both genotypes (Supplementary Fig. 4; CA1: for each group: n = 5, t (8) = 0.1808, p = 0.8610, p > 0.05; DG: for each group: n = 5, t (8) = 0.3357, p = 0.7457 > p > 0.05). Immunohistochemical analyses showed that CR and SST cell density in the mPFC, DLFC, and hippocampus was not affected by PlxnA1 deficiency. In the mPFC, DLFC, and hippocampus, there were no significant differences in CR cell density between WT and PlxnA1 KO mice (mPFC: Supplementary Fig. 5A, B, E; for each group: n = 5, t (8) = 0.24, p = 0.81, p > 0.05, DLFC: Supplementary Fig. 5C, D; for each group: n = 5, t (8) = 1.04, p = 0.32, p > 0.05, hoppocampus: Supplementary Fig. 6A, B, E; for each group: n = 5, t (8) = 0.29, p = 0.78, p > 0.05). Nor were there significant differences in SST cell density in the mPFC, DLFC, or hippocampus of PlxnA1 KO mice compared with WT (mPFC: Supplementary Fig. 7A, B, E; for each group: n = 5, t (8) = 0.94, p = 0.37, p > 0.05, DLFC: Supplementary Fig. 7C, D; for each group: n = 5, t (8) = 1.12, p = 0.29, p > 0.05, hippocampus: Supplementary Fig. 6C, D, F; for each group: n = 5, t (8) = 0.67, p = 0.52, p > 0.05).

4.4. Increased oxidative stress in PlxnA1 KO mice

In the mPFC, MICAL1 expression in neurons was visualized through antibody labeling against pan neuronal markers (Fig. 4A, B, C). 96% of MICAL1-positive cells in mPFC expressed pan neuronal markers (130 out of 135 cells with MICAL1; Fig. 4A, B, C). Thus, all neurons positive...
Fig. 2. Reduced cell density of GABA-positive cells in PtknA1 KO mice. Significantly decreased density of GABA-positive cells in the mPFC in PtknA1 KO mice (B) compared to WT (A); significant decrease of GABA-positive cells at higher magnification (C, D). No change in GABA-positive cell density in the DLFC between WT (E) and PtknA1 KO mice (F). Statistical analyses revealed a significant reduction in GABA-positive cell density in the mPFC of PtknA1 KO mice compared with WT (G), but not in the DLFC (H). WT: wild type mice, KO: PtknA1 KO mice. GABA + cells: GABA-positive cells. WT (n = 5); PtknA1 KO (n = 5). Blue color: DAPI for nuclear stain (A, B). Data are presented as mean ± SEM. PrL: prelimbic cortex, Scale bars: 100 µm.
Fig. 3. Significant reduction of PV cell numbers in the mPFC of PlxnA1 KO mice. Immunofluorescence images of PV cells stained with anti-PV antibody in the mPFC in WT (A, C) and PlxnA1 KO mice (B, D) showing significantly reduced PV cell density in PlxnA1-deficient mPFC compared with WT. In the DLFC, the density of PV cells between WT (E) and PlxnA1 KO (F) mice was unaltered. Statistical analyses revealed a significant reduction in PV cell density in the mPFC of PlxnA1 KO mice compared with WT (G), but not in the DLFC (H). WT: wild type mice, KO: PlxnA1 KO mice. WT (n = 6); PlxnA1 KO (n = 6). Blue color: DAPI for nuclear stain (A, B). Data are presented as mean ± SEM. PrL: prelimbic cortex, Scale bars: 100 µm.
for pan neuronal markers exhibited the expression of MICAL1. In the mPFC, PlxnA1 mRNA colocalized in most neurons expressing MICAL1 (Fig. 4D–F), in which 74% of MICAL1-expressing cells showed the expression of PlxnA1 mRNA (181 out of 244 MICAL1-positive cells). MICAL1 was also expressed in PV cells in the mPFC (Fig. 4G–I), in which 95% of PV cells showed the expression of MICAL1 (392 cells out of 413 PV cells). Since 42% of PV cells showed the expression of PlxnA1 mRNA in mPFC (Fig. 1C–F), PlxnA1 may be co-expressed with MICAL1 in approximately 40% of PV cells in the mPFC of WT. Therefore, the lack of PlxnA1 may affect MICAL1 activity, in turn leading to increased oxidative stress in PV cells in the mPFC of PlxnA1 KO mice. To examine if PV cells in the mPFC of PlxnA1 KO mice exhibit abnormal levels of oxidative stress compared with WT, double 8-ox-dG and PV immunostaining was performed in mice at 6 months of age. As a result, significantly higher fluorescence 8-oxo-dG intensity was observed in PV cells of the mPFC in PlxnA1 KO mice compared to WT (Fig. 5A–K, WT: n = 6, KO: n = 5, t (9) = 2.61, p = 0.028, p < 0.05). Thus, at 6 months of age, there was significantly higher oxidative stress in PV cells in the mPFC of PlxnA1 KO mice than in WT.

5. Discussion

The present study revealed PlxnA1 mRNA expression in PV cells in the mPFC and hippocampus of adult mice. Moreover, we detected a

Fig. 4. MICAL1 expression analysis. MICAL1 expression visualized in neuronal cell bodies by immunostaining of MICAL1 and pan neuronal marker proteins (A, B, C). PlxnA1 mRNA (D) and MICAL1 (E) co-expression revealed by in situ PlxnA1 hybridization followed by MICAL1 immunohistochemistry (F). PV (G) and MICAL1 (H) co-expression revealed by PV and MICAL1 immunostaining (I). PanN marker: pan neuronal marker proteins. Blue color: DAPI for nuclear stain (A, B). PrL: prelimbic cortex. Scale bars: 50 μm.
Fig. 5. Increased oxidative stress in PV cells of the mPFC in PlxnA1 KO mice. Immunofluorescence images of PV (red) and 8-oxo-dG (green) in the mPFC of WT (A, C, E: merged view) and PlxnA1 KO mice (B, D, F: merged view). At higher magnification, a significant elevation of 8-oxo-dG intensity in PV cells indicates oxidative stress in PV cells in the mPFC of PlxnA1 KO mice (J: arrows denote green 8-oxo-dG fluorescence in PlxnA1-deficient PV cells) compared with WT (I: arrows denote green 8-oxo-dG fluorescence in WT-PV cells). Higher 8-oxo-dG intensity in PV cells reveals a significantly elevated oxidative level in PV cells of the mPFC in PlxnA1 KO mice compared with WT (K). WT: wild type mice, KO: PlxnA1-KO mice. WT (n = 6); PlxnA1 KO (n = 5). Data are presented as mean ± SEM. PrL: prelimbic cortex. Scale bars: 50 µm.
significant reduction of both GABAergic neurons and PV cells in the mPFC of PlxnA1 KO mice than in WT. PlxnA1 mRNA appeared to be co-expressed with MICAL1 in PV cells of the mPFC of WT. Finally, we found significantly higher oxidative stress levels in PV cells in the mPFC of PlxnA1 KO mice compared with WT. Thus, the significant decrease in PV cell number and enhanced oxidative stress in such cells in the mPFC may influence the abnormal behavior of PlxnA1 KO mice.

PlxnA1 mRNA was expressed in PV cells in the mPFC and hippocampus of adult mice (Fig. 1D–F). Since most PV cells are enveloped by PNNs (Giamanco et al., 2010), this result is consistent with previous work which reported PlxnA1 and PlxnA4 expression in interneurons wrapped with PNNs in the cerebral cortex of adult mice (Vo et al., 2013). We detected significant reductions of GABAergic interneurons and PV cells in the mPFC of PlxnA1 KO mice compared with WT (Fig. 2A–D, G, Fig. 3A–D, G), but not in the DLFC (Fig. 2E, F, H, Fig. 3E, F, H) or hippocampus (Supplementary Fig. 1). Thus, among the brain regions examined in the present study, decreased GABAergic neuron and PV cell densities in PlxnA1 KO mice were detected only in the mPFC; moreover, only the subpopulation of PV cells showed decreased density. Andrews et al. (2016) reported significantly reduced proliferative ability of interneuron progenitors and interneuron density in the developing cerebral cortex of PlxnA1 KO mice at E14.5 and E18.5. We detected decreased PV cell density in the mPFC in PlxnA1 KO mice at 6 months of age, but not at 2 weeks of age. Thus, the PV cell density in the mPFC may be less influenced by the developmental defects of GABAergic interneurons during embryonic stage in PlxnA1 KO mice. Postnatal long-term effects of PlxnA1-deficiency in PV cells may gradually decrease PV cell density in the mPFC. The neocortex is engaged in the general modulation of self-grooming motor behavior by placing excitatory projections into the striatum which regulates motor behavior of self-grooming (Kaluuff et al., 2016). Mice with a glutamatergic Shank3 deletion with enhanced self-grooming display abnormally increased neuronal excitability in pyramidal neurons in mPFC (Yoo et al., 2019). Thus, the decrease of PV cells in mPFC may lead to the enhanced excitability in pyramidal neurons, by which excitatory projections to the striatum may be intensified and thus self-grooming may be increased (Kaluuff et al., 2016). The mPFC, nucleus accumbens, ventral hippocampus and ventral tegmental area are involved in the regulation of prepulse inhibition (Swerdlow et al., 2001). The mPFC sends an intense direct projection to the ventral hippocampus and nucleus accumbens (Gabbott et al., 2005). The mPFC also receives afferents from multiple cortical and subcortical areas like ventral hippocampus (Caballero et al., 2014). Furthermore, a 25% reduction of adult PV levels in the mPFC (Yoo et al., 2019). Thus, the reduction of PV cells in mPFC may lead to deficit of prepulse inhibition due to the impairment in mutual connection of mPFC and other areas crucial for prepulse inhibition.

There were no significant differences in cell density and superficial laminar distribution of PV cells in DLFC between both genotypes (Fig. 3F, H, Supplementary Fig. 3). Sema3A-PlxnA4 regulates the superficial laminar positioning of serotonin receptor 3a-expressing cortical interneurons (Limoni and Niquille, 2021; Limoni et al., 2021). PlxnA4 is also involved in promoting basal dendrite arborization of cortical neurons through the Sema3A-Nrp1/PlxnA4/FARP2/Rac1 signaling pathway (Danelon et al., 2020). PlxnA4 is involved in both cortical guidance of interneurons from medial ganglionic eminence (MGE) and perinatal positioning of serotonin receptor 3a-expressing cortical interneurons in superficial layers, and promotes in proliferation of interneuron progenitors in MGE and cortical plate (Limoni and Niquille, 2021; Andrews et al., 2016). Thus, PlxnA4 may be dispensable for the laminar positioning of PV cells in DLFC. Even though PlxnA1-deficiency may increase oxidative stress of PV cells in DLFC, PV cells in DLFC may be more resistant to high oxidative stress than those of mPFC. Since mPFC including anterior cingulate cortex receives projection of dopaminergic axons from the midbrain (Kolk et al., 2009), PlxnA1-deficient PV cells in mPFC may have higher oxidative stress due to their faster and higher frequent discharges than those in DLFC.

Our study revealed that MICAL1 localizes in PV cells expressing PlxnA1 mRNA (Fig. 4). MICAL1 mRNAs are ubiquitously expressed in the rat developing and adult nervous system (Pasterkamp et al., 2006). The immunohistochemical analysis of both WT and MICAL1−/− mice showed MICAL1 localization in granule cells of the dentate gyrus and in mossy fibers of the postnatal and adult hippocampus (Van Battum et al., 2014). Since MICAL1 tends to be expressed at higher levels in pan neuronal marker-positive neurons than in pan neuronal marker-negative cells (Fig. 4), MICAL1 may have a crucial role in the maintenance and functioning of neural circuits in the mPFC. Several studies have suggested the protective effect of MICAL1 in neuron development against neurological diseases with oxidative stress-associated pathogenesis (Xu et al., 2021; Li et al., 2019; Deng et al., 2016; Hung et al., 2010; Pasterkamp et al., 2006). Semaphorin 3 F (Sema3F) regulates MICALs’ activity through the interaction of MICALs with PlxnA, Sema3F receptor (Li et al., 2019). Interneuron-specific Sema3F KO mice exhibit less interneurons/GABAergic neurites, transcripts for GABAergic subtype markers (PV, Neurondentin Y), and increased epileptic seizure and autism-like behavior (Li et al., 2019). In addition, these KO mice showed significantly increased oxidative stress and neuroinflammation associated with microglial increase in the cerebral cortex, hippocampus, and amygdala, which may be caused by MICAL dysfunction (Li et al., 2019). The recent study of the interneuron-specific Sema3F KO mice indicates that production of reactive oxygen species (ROS) through microglial activation due to disruption of blood brain barrier (BBB) is associated with neuroinflammation, activation and extravascular deposition of platelets, serotonin secretion and albumin leakage into the brain leading to increased oxidative stress in the KO mice (Jagadapilli et al., 2022). Thus, another hypothesis for increased oxidative stress in PlxnA1 KO mice is that enhanced vascular permeability may induce the increase of oxidative stress, decrease of PV cells and abnormal behavior. Nrp1 is involved in regulating developmental angiogenesis and, vascular permeability and angiogenesis under various pathological states by functioning as a coreceptor for Sema3A (Domínguez and Fantin, 2021; Yang et al., 2019). Sema3A-induced endothelial cell permeability needs PlxnA1 to cause instability of interendothelial junctions integrity (Le Guelte et al., 2012). Thus, PlxnA1 deficiency may cause dysregulation of vascular permeability in the brain which may lead to increased vascular permeability, microglial activation, production of ROS and neuroinflammation. Neurophin 2 (Nrp2)-deficient mice display similar phenotypes with the interneuron-specific Sema3F KO mice in behavioral, cell morphological and immunohistochemical studies (Eisenberg et al., 2021; Assous et al., 2019; Shiflett et al., 2015; Gant et al., 2009). The first report showed significant reduction of GABA, PV and Neuropetide Y (NPY)-expressing interneurons without any decrease of SST cells in hippocampus of Nrp2 KO mice (Gant et al., 2009). Another paper reported significant decrease of SST cells in all hippocampal subregions and selective reduction of PV cells and NPY cells in CA1 of Nrp2 KO mice (Eisenberg et al., 2021). Both studies discuss the possibility of impairment in the developmental migration of interneurons. Since Nrp2 functions as a co-receptor for Sema3F and interneuron-specific Sema3F KO mice exhibit the decrease of interneuron number in hippocampus (Li et al., 2019), the decreased interneuron density in hippocampus of Nrp2 KO mice may be in part caused by increased oxidative stress, microglial activation, neuroinflammation and so on due to the disruption of blood brain barrier (Jagadapilli et al., 2022; Li et al., 2019). In another study, blood-mediated MICAL1 knockdown decreased the expression of nuclear factor erythroid 2-related factor 2 and both anti-apoptotic factor and autophagy-related proteins, and increased the expression of pro-apoptotic factors in oligodendrocytes cultured with hydrogen peroxide in an in vitro spinal cord injury model (Xu et al., 2021). Accordingly, MICAL1 dysregulation induced by Sema3F or Nrp2 or PlxnA deficiency may increase oxidative stress in neurons and glia leading to disease exacerbation.

The present study revealed increased oxidative stress in PV cells of
the mPFC in PlxnA1 KO mice at 6 months of age. The lack of PlxnA1 may increase DNA oxidation through dysregulated MICAL1 activity in PC cells in the mPFC. In animal models of neuropsychiatric disorders such as schizophrenia and Fragile X syndrome with autism spectrum disorders, increased oxidative stress in PC cells is a common factor impairing both PC cell and PNN integrity (Steullet et al., 2017; Cabungcal et al., 2014). Increased oxidative stress affects the normal phenotype of PC cells, including the lower PV immunoreactivity (Powell et al., 2012; Behrens et al., 2007). Thus, the increased oxidative stress in PC cells may decrease their density in the mPFC in PlxnA1 KO mice at 6 months of age. PC cells promote information processing through synchronization of neural activities among brain regions by controlling the neuronal firing of neighboring pyramidal neurons (Lewis et al., 2012; Whittington et al., 2011; Sohal et al., 2009). Accordingly, the decreased PV cell density in the mPFC of PlxnA1 KO mice may cause deficient neural information processing and lead to abnormal behaviors reported previously in this KO mice, including prepuIse inhibition deficit, excessive repetitive self-grooming behavior, and reduced anxiety (Jahan et al., 2020).

In the pathway from the increased oxidative stress leading to abnormal behavior, crucial points: enhanced oxidative stress in PC cells, decreased PC cell density in the mPFC, and abnormal behaviors in PlxnA1 KO mice have been demonstrated in our study. However, the detailed mechanism causing increased oxidative stress in PlxnA1-deficient PC cells remains unknown. It also remains unclear as to whether increased oxidative stress in PC cells is a primary cause of the abnormal behavior. Future work addressing these problems with various experiments such as antioxidant treatment (Cabungcal et al., 2014) is required to fully understand the pathogenesis of abnormal behaviors in PlxnA1 KO mice.

6. Conclusion

Our study revealed PlxnA1 mRNA expression in PC cells in the mPFC of adult mice. Moreover, a significant reduction of PC cells was detected in the mPFC of PlxnA1 KO mice than in WT. Finally, significantly higher oxidative stress in PC cells was found in the mPFC of PlxnA1 KO mice compared with WT. Thus, enhanced oxidative stress in PC cells and the significant decrease in PC cell density in the mPFC may be decisive factors for the onset of the abnormal behavior of PlxnA1 KO mice.

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CRediT authorship contribution statement

Mst Sharifa Jahan: Investigation, Writing – original draft. Takamasa Tsuzuki: Investigation, Methodology, Supervision. Takuji Ito: Investigation, Methodology. Md. Elissur Rahman Bhuiyan: Investigation, Methodology. Hyota Takamatsu: Resources. Atsushi Kumanogoh: Resources. Takayuki Negishi: Investigation, Methodology, Supervision. Kazunori Yukawa: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Project administration, Software, Supervision, Validation, Visualization, Writing – review & editing.

Conflict of interest

None.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ibneur.2022.11.002.

References

Aika, Y., Ren, J.Q., Konsaka, K., Konsaka, T., 1994. Quantitative analysis of GABA-like immunoreactive and parvalbumin-containing neurons in the CA1 region of the rat hippocampus using a stereological method, the disector. Exp. Brain Res. 99, 267–276. https://doi.org/10.1007/BF00239593.

Alto, L.T., Terman, J.R., 2018. M1CAbs. Curr. Biol. 28, R538–R541. https://doi.org/10.1016/j.cub.2018.01.025.

Andrews, W.D., Davidson, K., Tamamaki, N., Ruberg, C., Parnavelas, J.G., 2016. Altered proliferative ability of neuronal progenitors in PlxnA1 mutant mice. J. Comp. Neurol. 524, 518–534. https://doi.org/10.1002/cne.23806.

Andrews, W.D., Barber, M., Nemitz, M., Memi, F., Parnavelas, J.G., 2017. Semaphorin3A-neuropilin1 signalling is involved in the generation of cortical interneurons. Brain Struct. Func. 222, 2217–2233. https://doi.org/10.1007/s00381-016-1372-5.

Assous, M., Martinez, E., Eisenberg, C., Shah, F., Kosc, A., Varghese, K., Espinoza, D., Bhimani, S., Tepper, J.M., Shiflett, M.W., Tran, T.S., 2019. Neuropilin 2 signaling mediates corticostriatal transmission, spine maintenance, and goal-directed learning in mice. J. Neurosci. 2019 (39), 8845–8859. https://doi.org/10.1523/JNEUROSCI.0706-19.2019.

Behrens, M.M., Ali, S.S., Dao, D.N., Lucero, J., Shekhtman, G., Quick, K.L., Dugan, L.L., 2007. Ketamine-induced loss of phenotype of fast-spiking interneurons is mediated by NADPH-oxidase. Science 318, 1645–1647. https://doi.org/10.1126/science.1148045.

Ben-Ari, Y., 2014. The GABA excitatory/inhibitory developmental sequence: a personal journey. Neuroscience 279, 187–219. https://doi.org/10.1016/j.neuroscience.2014.08.001.

Boggio, E.M., Eldert, E.M., Lupori, L., Moloney, E.B., De Winter, F., Vander Kooi, C.W., Baronecelli, M., Mecollari, V., Blits, B., Fawcett, J.W., Verhaagen, J., Pizzorusso, T., 2019. Inhibition of semaphorin3A promotes ocular dominance plasticity in the adult rat visual cortex. Mol. Neurobiol. 2019 (56), 5987–5997. https://doi.org/10.1007/s12035-019-1499-0.

Bray, N.J., O’Donovan, M.C., 2019. The genetics of neuropsychiatric disorders. Brain Neurosci. Adv. 2 https://doi.org/10.1523/1084-016-2018.12.001.

Caballero, A., Thomasae, D.B., Flores-Barrera, E., Cas, D.K., Tseng, K.Y., 2014. Downregulation of parvalbumin expression in the prefrontal cortex during adolescence. Psychopharmacology 231, 1789–1796. https://doi.org/10.1007/s00213-013-3216-4.

Caballero, A., Flores-Barrera, E., Thomasae, D.B., Tseng, K.Y., 2020. Downregulation of parvalbumin expression in the prefrontal cortex during adolescence causes enduring prefrontal disinhibition in adulthood. Neuropsychopharmacology 45, 1527–1535. https://doi.org/10.1038/s41386-020-0799-9.

Cabungcal, J.H., Couteau, D.S., Lewis, E., Tejeda, H.A., Piantadosi, P., Pollock, C., Calhoon, G.G., Sullivan, E., Prengraves, E., Kil, J., Hong, L.E., Cuenod, M., Do, K.Q., O’Donnell, P., 2014. Juvenile antiepileptic treatment prevents adult deficits in a developmental model of schizophrenia. Neuron 83, 1073–1084. https://doi.org/10.1016/j.neuron.2014.07.028.

Carulli, D., Verhaagen, J., 2021. An extracellular perspective on CNS maturation: perineuronal nets and the control of plasticity. Int. J. Mol. Sci. 22, 4243. https://doi.org/10.3390/ijms22054243.

Chedele, L., Biederer, T., 2014. Activity-dependent regulation of dendritic complexity by semaphorin 3A through Farp1. J. Neurosci. 34, 7999–8009. https://doi.org/10.1523/JNEUROSCI.3543-13.2014.

Danelon, V., Golden, R., Martinez, E., Goldman, I., Wang, K., Yaron, A., Tran, T.S., 2020. Modular and distinct plexin-A4/FARP2/Rac1 signaling controls dendrite morphogenesis. J. Neurosci. 40, 5413–5430. https://doi.org/10.1523/NEUROSCI.2730-19.2020.

Deboyre-Bourgeois, C., Jacquier, A., Charuy, C., Reynaud, F., Nawabi, H., Thionet, K., Kindbeiter, K., Yoshida, Y., Zagor, K., Yang, J., Jones, Y.E., Falk, J., Chedotad, A., Castellani, V., 2015. FlexinA1 is a new Slit receptor and mediates axon guidance function of Slit-Cterminal fragments. Nat. Neuroscience. 18, 1084–1085. https://doi.org/10.1038/nn.3993.

Deng, W., Wang, Y., Gu, L., Duan, B., Cui, J., Zhang, Y., Chen, Y., Sun, S., Dong, J., Du, J., 2016. MICAl1 controls cell invasive phenotype via regulating oxidative stress in breast cancer cells. BMC Cancer 16, 489. https://doi.org/10.1186/s12885-016-2553-1.

Domingues, A., Fantin, A., 2021. Neuropilin 1 regulation of vascular permeability signaling. Biomolecules 11, 666. https://doi.org/10.3390/biom11050666.

Duan, Z.R.S., Che, A., Chu, P., Modoi, L., Bollmann, Y., Babj, R., Fetcho, R.N., Otsuka, T., Fuccillo, M.V., Liston, C., Piaap, D.J., Consart, R., De Marco Garcia, N.V., 2020.
Sigurdsson, T., Duvarci, S., 2015. Hippocampal-prefrontal interactions in cognition, behavior and psychiatric disease. Front. Syst. Neurosci. 9, 190. https://doi.org/10.3389/fnsys.2015.00190.

Sohal, V.S., Zhang, F., Vizhar, O., Deisseroth, K., 2009. Parvalbumin neurons and gamma rhythms enhance cortical circuit performance. Nature 459, 698-702. https://doi.org/10.1038/nature07991.

Steullet, P., Cabungcal, J.H., Coye, J., Didrikson, M., Gill, K., Grace, A.A., Hench, T.K., LaManita, A.S., Lindemann, L., Maynard, T.M., Meyer, U., Morishita, H., O’Donnell, P., Puhl, M., Cuenod, M., Do, K.Q., 2017. Oxidative stress-driven parvalbumin interneuron impairment as a common mechanism in models of schizophrenia. Mol. Psychiatry 22, 936-943. https://doi.org/10.1038/mp.2017.47.

Sun, Q., Li, X., Li, A., Zhang, J., Ding, Z., Gong, H., Luo, Q., 2020. Ventral hippocampal-parietal interaction affects social behavior via parvalbumin positive neurons in the medial prefrontal cortex. Science 23, 108094. https://doi.org/10.1016/j.sci.2020.108094.

Swerdlow, N.R., Geyer, M.A., Braff, D.L., 2001. Neural circuit regulation of prepulse inhibition of startle in the rat: current knowledge and future challenges. Psychopharmacology 156, 194-215. https://doi.org/10.1007/s002130100799.

Takegahara, N., Takamatsu, H., Toyofuku, T., Tsujimura, T., Okuno, T., Yukawa, K., Mizui, M., Yamamoto, M., Prasad, D.V.R., Suzuki, K., Ishii, M., Terai, K., Moriya, M., Nakatsuji, Y., Sakoda, S., Sato, S., Akira, S., Takeda, K., Inui, M., Takai, T., Ikawa, M., Okabe, M., Kumanogoh, A., Kikutani, H., 2006. Plexin-A1 and its interaction with DAP12 in immune responses and bone homeostasis. Nat. Cell Biol. 8, 615-622. https://doi.org/10.1038/ncc1416.

Tamamaki, N., Yasagawa, Y., Tomioka, R., Miyazaki, J., Obata, K., Kameko, T., 2003. Green fluorescent protein expression and colocalization with calretinin, parvalbumin, and somatostatin in the GAD67-GFP knock-in mouse. J. Comp. Neurol. 467 (1), 60-79. https://doi.org/10.1002/cne.10905.

Terman, J.R., Mao, T., Pasterkamp, R.J., Yu, H.H., Kolodkin, A.L., 2002. MICAla, a family of conserved flavoprotein oxidoreductases, function in plexin-mediated axonal repulsion. Cell 109, 887-900. https://doi.org/10.1016/s0092-8674(02)00794-8.

Van Battum, E.Y., Gunput, R.A., Lemstra, S., Groen, E.J., Yu, K.L., Adolfs, Y., Zhou, Y., Takegahara, N., Takamatsu, H., Toyofuku, T., Tsujimura, T., Okuno, T., Yukawa, K., Mizui, M., Yamamoto, M., Prasad, D.V.R., Suzuki, K., Ishii, M., Terai, K., Moriya, M., Nakatsuji, Y., Sakoda, S., Sato, S., Akira, S., Takeda, K., Inui, M., Takai, T., Ikawa, M., Okabe, M., Kumanogoh, A., Kikutani, H., 2006. Plexin-A1 and its interaction with DAP12 in immune responses and bone homeostasis. Nat. Cell Biol. 8, 615-622. https://doi.org/10.1038/ncc1416.

Voigt, T., Opitz, T., De Lima, A.D., 2001. Synchronous oscillatory activity in immature hippocampal mossy fibre connections. Nat. Commun. 5, 4317. https://doi.org/10.1038/ncb1416.

Vo, T., Caralli, D., Ehlert, E.M., Kwoo, J.C., Dick, G., Mecollari, V., Moloney, E.B., Neufeld, G., de Winter, F., Fawcett, J.W., Verhaagen, J., 2013. The chemorepellive axon guidance protein semaphorin3A is a constituent of perineuronal nets in the adult rodent brain. Mol. Cell. Neurosci. 56, 194-215. https://doi.org/10.1016/j.mcn.2013.04.005.

Vo, T., Caralli, D., Ehlert, E.M., Kwoo, J.C., Dick, G., Mecollari, V., Moloney, E.B., Neufeld, G., de Winter, F., Fawcett, J.W., Verhaagen, J., 2013. The chemorepellive axon guidance protein semaphorin3A is a constituent of perineuronal nets in the adult rodent brain. Mol. Cell. Neurosci. 56, 186–200. https://doi.org/10.1016/j.mcn.2013.04.005.

Voigt, T., Opitz, T., De Lima, A.D., 2001. Synchronous oscillatory activity in immature cortical network is driven by GABAergic preplate neurons. J. Neurosci. 21, 8895-8905. https://doi.org/10.1523/jneurosci.21-22-08895.2001.

Wainey, K.-E., Cheng, H.J., 2006. Axon pruning and synaptic development: how are they per-plexin? Neuroscientist 12, 398-409. https://doi.org/10.1177/1073858406292631.

Whittington, M.A., Cunningham, M.O., LeBeau, F.E., Racca, C., Traub, R.D., 2011. Multiple origins of the cortical γ rhythm. Dev. Neurosci. 37, 91–106. https://doi.org/10.1002/dneu.20814.

Winberg, M.L., Noordermeer, J.N., Tamagnone, L., Comoglio, P.M., Spiggs, M.K., Tesnier-Lavigne, M., Goodman, C.S., 1998. Plexin A is a neuronal semaphorin receptor that controls axon guidance. Cell 95, 903–916. https://doi.org/10.1016/s0092-8674(00)81715-8.

Xu, C., Mao, L., Tian, H., Lin, S., Zhao, X., Lin, J., Li, D., Li, X., Mei, X., 2021. MICAL1 (molecule interacting with Casl.1) protects oligodendrocyte cells from oxidative injury through regulating apoptosis, autophagy in spinal cord injury. Neurosci. Lett. 750, 135712 https://doi.org/10.1016/j.neulet.2021.135712.

Xu, X., Roby, K.D., Callaway, E.M., 2010. Immunohistochemical characterization of inhibitory mouse cortical neurons: three chemically distinct classes of inhibitory cells. J. Comp. Neurol. 518, 389-404. https://doi.org/10.1002/cne.22229.

Yang, M., Wang, X., Fan, Y., Chen, Y., Sun, D., Xu, X., Wang, J., Gu, G., Peng, R., Shen, T., Liu, X., Li, F., Wang, Y., Wang, D., Rong, H., Han, Z., Gao, X., Li, Q., Fan, K., Yuan, Y., Zhang, J., 2019. Semaphorin 3A contributes to secondary blood-brain barrier damage after traumatic brain injury. Front Cell Neurosci. 13, 117. https://doi.org/10.3389/fncel.2019.00117.

Yoo, T., Cho, H., Park, H., Lee, J., Kim, E., 2019. Shank3 exons 14-16 deletions in glutamatergic neurons leads to social and repetitive behavioral deficits associated with increased cortical layer 2/3 neuronal excitability. Front. Cell Neurosci. 13, 458. https://doi.org/10.3389/fncel.2019.00458.

Zhao, X.F., Kohen, R., Parent, R., Duan, Y., Fisher, G.L., Korn, M.J., Ji, L., Wan, G., Jin, J., Pischel, A.W., Dolan, D.F., Parent, J.M., Corfas, G., Murphy, G.G., Giger, R.J., 2018. PlexinA2 forward signaling through Rap1 GTPases regulates dentate gyrus development and schizophrenia-like behaviors. Cell Rep. 22, 456-470. https://doi.org/10.1016/j.celrep.2017.12.044.