IBRO Reports 9 (2020) 276–289

Contents lists available at ScienceDirect

IBRO Reports

journal homepage: www.journals.elsevier.com/ibro-reports

PlexinA1 deficiency in BALB/cAJ mice leads to excessive self-grooming and reduced prepulse inhibition

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ARTICLE INFO

Keywords:
Semaphorin
Plexin
Grooming
Prepulse inhibition
Neurodevelopmental disorder
Psychiatric disorder

ABSTRACT

PlexinA1 (PlxnA1) is a transmembrane receptor for semaphorins, a large family of proteins that act as axonal guidance cues during nervous system development. However, there are limited studies on PlxnA1 function in neurobehavior. The present study examined if PlxnA1 deficiency leads to behavioral abnormalities in BALB/cAJ mice. PlxnA1 knockout (KO) mice were generated by homologous recombination and compared to wild type (WT) littermates on a comprehensive battery of behavioral tests, including open field assessment of spontaneous ambulation, state anxiety, and grooming, home cage grooming, the wire hang test of muscle strength, motor coordination on the rotarod task, working memory on the Y maze alternation task, cued and contextual fear conditioning, anxiety on the elevated plus maze, sociability to intruders, and sensory processing as measured by prepulse inhibition (PPI). Measures of motor performance, working memory, fear memory, and sociability did not differ significantly between genotypes, while PlxnA1 KO mice displayed excessive self-grooming, impaired PPI, and slightly lower anxiety. These results suggest a crucial role for PlxnA1 in the development and function of brain regions controlling self-grooming and sensory gating. PlxnA1 KO mice may be a valuable model to investigate the repetitive behaviors and information processing deficits characteristic of many neurodevelopmental and psychiatric disorders.

1. Introduction

During development of the embryonic nervous system, growing axons are directed toward their targets by multiple extracellular guidance molecules that interact with specific receptor proteins in the growth cone membrane (Tessier-Lavigne and Goodman, 1996). The mammalian nervous system expresses numerous attractive or repulsive axon guidance molecules in the extracellular environment, including a large family of semaphorins (Semas) (Goodman et al., 1999) that interact with growth cone receptors termed Plexins (Plxns) (Alto and Terman, 2017). Nine *Plxn* genes have been identified in the mammalian genome and classified into four subfamilies, *PlxnA1−4*, *PlxnB1−3*, *PlxnC1*, and *PlxnD1* (Raper, 2000; Negishi et al., 2005). Each Plxn receptor contains a Sema domain composed of ~500 amino acids at the amino (N)-terminal region of the extracellular portion that binds Sema dimers as well as a GTPase-activating protein (GAP) domain in the intracellular region for signal transduction (Liu et al., 2010; Tamagnone et al., 1999). While plexins act as the main receptors for the intracellular signal transmission of Semas, several other membrane-bound proteins also serve as Sema receptors or co-receptors (Toyofuku et al., 2004; Takegahara et al., 2006; Bouvrée et al., 2012; Jongbloets and Pasterkamp, 2014; Worzfeld and Offermanns, 2014), including neuropilins (Nrps), which act in conjunction with PlxnA1 in the ligand-binding subunit for class 3 Semas, PlxnA1 intracellularly transmits signals for class 3 Semas, a group of repulsive axon guidance molecules (Takahashi et al., 1999).

PlxnA1 is widely expressed throughout the developing nervous systems, including the auditory system, olfactory system, neocortex,

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https://doi.org/10.1016/j.ibror.2020.10.004
Received 10 August 2020; Accepted 20 October 2020
Available online 22 October 2020

hippocampus, neural retina, and peripheral ganglia (Murakami et al., 2001; Perälä et al., 2005), indicating multiple critical functions in forebrain development (Murakami et al., 2001; Kameyama et al., 1996). Indeed, in a PlxnA1-deficient mouse line on the C57BL/6J background, neuronal progenitor cells of the medial ganglionic eminence were poorly attached to the ventricular surface and displayed reduced proliferative capacity (Andrews et al., 2016), resulting in impaired GABAergic neurogenesis in the embryonic cortex and reduced numbers of striatal projection neurons (Andrews et al., 2016). PlxnA1 also acts as a crucial receptor for guidance cues for the navigation of callosal axons (Wu et al., 2014). A PlxnA1 KO mouse line on the BALB/cAJ background exhibited a significantly lower rate of midline crossing of callosal axons compared to WT controls during the embryonic stage, indicating involvement in corpus callosum (CC) formation (Hossain et al., 2019).

Recent genetic studies have implicated Sema–Plxn pathway malfunction in numerous brain disorders including schizophrenia (Fujii et al., 2011; Mah et al., 2006; Gilabert-Juan et al., 2015), autism (Weiss et al., 2009), Alzheimer's disease (Jun et al., 2014; Wang et al., 2016), and Parkinson's disease (Schulte et al., 2013). For instance, an adult human with symptoms of schizophrenia was shown to harbor a missense *PlxnA1* variant (NM_032242, c.4201A>T, p.1401M>L) (Fromer et al., 2014), while a case of epileptic encephalopathy harbored a missense *PlxnA1* mutation (NM_032242.2:c.683G>A, p.Arg228His) (Epi4K Consortium et al., 2013; Oliver et al., 2016). Another case presenting with developmental encephalopathy characterized by intractable epilepsy of infantile-onset, frequent hyperkinetic movement, stereotypies, autism, and dysmorphic facial features indicative of Dubowitz syndrome demonstrated a de novo heterozygous variant of *PlxnA1* (NM_032242, chr3:g.126735788, c.3184G>A, p.Gly1062Ser) (Park et al., 2017). Such findings suggest that *PlxnA1* allelic variants and mutations may cause heterogeneous morphological, neurofunctional, and behavioral abnormalities, consistent with multiple functions in neurodevelopment across brain regions.

The prominent expression of PlxnA1 in the mouse forebrain during development and these relationships with several neurodevelopmental and neuropsychiatric disorders prompted our present analysis of PlxnA1 functions in the complex behaviors of adult mice.

2. Material and methods

2.1. Animals

PlxnA1 knockout (KO) mice on the BALB/cAJ genetic background were generated by gene targeting (Takegahara et al., 2006). In brief, a gene targeting vector was prepared by replacing the genomic region containing the initiation codon and Sema domain-coding sequence with a neomycin resistance gene. The vector was then transfected into E14.1 embryonic stem (ES) cells by electroporation. G418- and ganciclovir-resistant clones were screened by polymerase chain reaction (PCR) and confirmed by Southern blotting. Mutant ES cells were introduced into mouse blastocysts, and the blastocysts were transferred into uteri of pseudopregnant mice to generate chimeras. F1 heterozygous mice were produced by mating the chimeras with BALB/cAJ mice and then backcrossed with BALB/cAJ mice for 10 generations. Pairs of the resultant heterozygous mice were bred to generate homozygous KO mice and wild type (WT) littermates as controls.

A GAD67-green fluorescent protein (GFP) knock-in mouse (ICR.Cg-Gad1 < tm1.1Tama>) was provided by the RIKEN BRC through the National Bio-Resource Project of the MEXT, Japan (Tamamaki et al., 2003) and backcrossed with BALB/cAJ mice for 10 generations to establish a BALB/cAJ line with fluorescent marking of GABA neurons.

All mice were raised in the animal center of the Faculty of Pharmacy, Meijo University. WT and PlxnA1 KO littermates were housed in groups of six with ad libitum access to water and food (MM-3, Sankyo Labo Service Corporation, INC., Tokyo, Japan) under a controlled environment with 50.5% humidity, 23.1 °C ambient temperature, 300 lx illuminance, and a 12 h/12 h light/dark cycle (lights on at 8:00). All animal care and experimental procedures were conducted 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).

2.2. Genotype analysis

Genotyping of both WT and PlxnA1 KO mice was performed by PCR using mouse tail DNA as the template and a *PlxnA1* gene-specific primer set as previously reported (Ito et al., 2014; Takegahara et al., 2006). Genotyping of the GAD67-GFP knock-in mouse was performed following the procedure in the original report (Tamamaki et al., 2003).

2.3. Western blotting

Brain tissues were homogenized in T-PER Tissue Protein Extraction Reagent containing 25 mM bicine and 150 mM sodium chloride (pH 7.6) (Thermo Fisher Scientific Inc. MA, USA), cOmplete ULTRA Tablets, Mini, *EASYpack* Protease Inhibitor Cocktail (05892970001, Roche Applied Science, Penzberg, Germany), and PhosStop (4906845001, Roche Applied Science). The homogenates were centrifuged at 10,000 rpm for 10 min at 4 °C to obtain the soluble protein fraction. Supernatant protein concentration was determined using a bicinchoninic acid (BCA) protein assay kit (Thermo Fisher Scientific Inc.). Proteins were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and semiquantified by western blotting as previously described (Hossain et al., 2019). Briefly, separated proteins were electrotransferred to polyvinylidene difluoride (PVDF) membranes and labeled with primary antibodies against β-Actin (1:10000, 13E5, Cell Signaling Technology, Danvers, MA, USA) and mPlexinA1 (1:5000, AF4309, R & D Systems, Minneapolis, USA) in Can Get Signal immunoreaction enhancer solution-1 (NKB-101, Toyobo Co. Ltd., Osaka, Japan). The membranes were then incubated with a horseradish peroxidase (HRP)-conjugated secondary antibody in Can Get Signal immunoreaction enhancer solution-2 (NKB-101, Toyobo Co. Ltd.) for 1 h at room temperature, followed by visualization using ECL™ prime reagents (GE Healthcare, Piscataway, NJ, USA). Analyses of protein band density were performed using Image Quant LAS-4000 (GE Healthcare Biosciences, Sweden).

2.4. In situ hybridization analysis of PlxnA1

Coronal sections of mouse brain were cut at 20 μm on a CryoStar NX70 cryostat (Thermo Scientific, Yokohama, Japan) and mounted on SuperFrost Plus slides (Fisher Scientific #12-550-15, MA, USA). In situ hybridization was performed on the sections using RNAscope Probes (Advanced Cell Diagnostics, CA, USA) targeting the 1091–1984 base pair region of the mouse *PlxnA1* gene (Accession No: NM_008881.2) according to the manufacturer's protocol. After in situ hybridization, sections were incubated with anti-GFP chicken polyclonal antibody (1:1000, ab13970, Abcam, Cambridge, UK) overnight at 4 °C. On the second day, the samples were washed and incubated with Alexa Flour 488 goat anti-chicken secondary antibody (1:1000, A11039, Thermo Fisher Scientific) for 1 h at room temperature. After incubation, sections were washed again and sealed under glass cover slips (Matsunami Glass Industries Ltd., Osaka, Japan) using Fluoro-KEEPER Antifade Reagent, Non-Hardening type containing DAPI for nuclear counterstaining (cat no. 12745-74, Nacalai Tesque Inc. Kyoto, Japan). The slices were imaged using the All-in-One Fluorescence Microscope (BZ-X710; Keyence, Osaka, Japan) controlled by BZ-X viewer version 1.3.1.1. Images were analyzed using BZ-X Analyzer version 1.4.0.1.

2.5. Behavioral tests

The open field test was conducted at 2, 4, and 6 months of age, while all other interventional behavioral tasks were conducted once at 6 months of age. The room for behavior tests was kept at 50.5% humidity, 23.1 °C, and 30 lx illuminance under a 12 h/12 h light/dark cycle (lights on at 8:00). Adult mice in standard mouse cages (27 cm length × 17 cm wide × 13 cm height) were acclimated to the test room for three days before behavioral testing to mitigate the effects of stress due to environmental changes. After every session, each apparatus was cleaned thoroughly with water and 70% alcohol to remove any dirt and odor cues.

2.5.1. Wire hang test

The wire hang test was conducted by placing the animal on a wire cage lid, then inverting the lid above a pad for 60 s. The latency of the animal to fall from the cage lid to the pad was measured as an index of limb muscle strength. A total of 22 WT and 18 KO mice were tested.

2.5.2. Rotarod test

Motor coordination and learning were examined using the standard and accelerating rotarod tests, respectively. In the first test, mice were placed on a drum (Ugo Basile; Gemonio, Varese, Italy) rotating at 4 rpm. The latency to fall off was recorded as an indicator of motor coordination. One week later, each mouse was again placed on the drum and rotation speed was gradually increased from 4 to 20 rpm over 5 min. The latency to fall off was recorded once a day for 3 consecutive days to measure motor learning. A total of 32 WT and 15 PlxnA1 KO mice completed both rotarod tasks.

2.5.3. Open field test

At 2, 4, and 6 months of age, each mouse was placed in the center of an open field apparatus (50 × 50 × 40 cm) and allowed to explore the environment freely for 20 min. Total distance traveled, number of intrusions into the central zone (central zone entries), time spent in the central zone, and the number and duration of grooming episodes were recorded and analyzed by ANY-MAZE (Stoelting Co.). A total of 23 WT and 21 PlxnA1 KO mice completed the three successive open field tests.

2.5.4. Grooming in the home cage

Grooming behavior in the home cage was also observed in a separate cohort of WT and PlxnA1 KO mice. Each mouse was housed individually in a standard mouse cage for behavioral analysis on day 1 and reared in the room for three days. The total durations of grooming and scratching from 20:00 to 8:00 on day 3 were analyzed using ANY-MAZE (Stoelting Co.). A total of 20 WT and 25 PlxnA1 mice were examined.

2.5.5. Prepulse inhibition (PPI)

Prepulse inhibition (PPI) was measured using the SR-Lab System (San Diego Instruments, San Diego, CA, USA). The system includes an acrylic measuring tube (3.8 cm in diameter and 13 cm in length) installed in a soundproof chamber and a vibration sensor connected to a signal amplification sensor. Each session started with 10 min of acclimation under 70-dB background noise, followed by both startle response and PPI trials. Each trial was presented ten times (total 40 times) in a randomized order. The sound stimulus protocols were (1) pulse alone (120 dB), (2) prepulse (74 dB) + pulse (120 dB), (3) prepulse (78 dB) + pulse (120 dB), and (4) prepulse (86 dB) + pulse (120 dB). The prepulse sound preceded the onset of the 120-dB startle stimulus by 100 ms. Percent (%)PPI was calculated as 100 − [(startle response on prepulse plus pulse trials/startle response on pulse alone trials) × 100]. Auditory startle reflex data were compared by Student's *t*-test and %PPI data by two-way ANOVA (factors genotype × prepulse sound level) with repeated measures. In total, 34 WT and 30 PlxnA1 KO mice completed all trials.

2.5.6. Elevated plus maze test

The elevated plus maze test was performed on 14 WT and 14 PlxnA1 KO mice following a method previously established (Boyce-Rustay and Holmes, 2006). Time spent on the open and closed arms were measured using a computer-assisted video tracking system (ANY-MAZE, Stoelting Co., Wood Dale, IL, USA).

2.5.7. Social interaction test

A three-chamber social interaction test was performed using a method modified from Silverman et al. (2010a). Identical columnar wire cages (diameter: 9 cm, height: 13.5 cm) were placed at equivalent positions in the left and right chambers of a three-chamber Plexiglass box (64 × 41.5 × 22.5 cm). On trial one, each mouse was placed in the central chamber and allowed to freely explore all three compartments for 10 min, and the time interacting with each empty cage was measured. The mouse was returned to the home cage, and an unfamiliar sex-matched BALB/cAJ WT mouse (Stranger 1) was put into one of the cages. In trial 2, the test mouse was placed back into the chamber and the time interacting with each cage was again recorded for 10 min (trial 2). After trial 2, the mouse was again returned to the home cage, and a novel mouse (Stranger 2) was introduced into the empty cage. In trial 3, the interaction times with Stranger mouse 1 and 2 were measured over 10 min. The interaction time with Stranger 1 in trial 2 was used as an index for sociability, while the interaction time with Stranger 2 in trial 3 was measured as an index of social novelty and memory. In total, 14 WT and 14 PlxnA1 KO completed all three trials.

2.5.8. Y maze spontaneous alternation test

The Y maze spontaneous alternation test was conducted following the method of Yamada et al. (1999). The Y maze used in this study is composed of three arms (43 cm long, 25 cm high, and 4 cm wide) projecting from a central triangular area. Mice were placed in the central area and allowed to explore freely for 5 min. The observer recorded an arm entry when the hind paws were completely within the arm. Spontaneous alternation was defined as successive entries into the three different arms (i.e., without returning to any arm). The percentage alternation was calculated as the ratio of actual to possible alternations (the total number of arm entries − 2) × 100. In total, 14 WT and 14 PlxnA1 KO mice completed the 5-min trial.

2.5.9. Fear conditioning

On the training day, each mouse was placed in a conditioning chamber (25.5 × 32 × 25.5 cm) equipped with a speaker and stainless steel grid floor connected to a shock source. Six pairings of a conditioned stimulus (CS: 80 dB, 15 s) followed by a mild foot shock (0.4 mA, 2 s) as an unconditioned stimulus (US) were delivered at 60-s intervals. A contextual fear conditioning test was conducted 24 hours after training in the same chamber for 3 min, during which the percentage time freezing (absence of all movement except from respiration) was measured. Mice were returned to home cage for 30 min. Each mouse was then placed in the chamber with a novel odor (soap) and exposed to the 80-dB cue alone for 60 s. Freezing time was measured during tone delivery. Freezing was recorded and analyzed automatically using VIDEO FREEZE SOFTWARE (Brain Science-idea.co., Osaka, Japan). A total of 14 WT and 14 PlxnA1 KO mice were tested under both contextual and cued conditions.

2.6. Assessment of agenesis of the corpus callosum by immunohistochemistry

One day after the final behavior tests at 6 months of age, mice received transcardiac perfusion of ice-cold PBS followed by ice-cold 4% paraformaldehyde (PFA) in PBS. The brain was quickly removed, post-fixed in 4% PFA solution overnight, cryoprotected by sequential immersion in 10%, 20%, and 30% sucrose solution, embedded in O.C.T. Compound, Tissue-Tek (Sakura Finetek Japan Co. Ltd., Tokyo, Japan),

promptly frozen on dry ice, and preserved at – 80 °C. Coronal slices of 10 μm thickness were prepared using a CryoStar NX70 cryostat (Thermo Scientific, Yokohama, Japan), mounted on glass slides (MAS-05, Matsunami Glass Industries Ltd., Osaka, Japan), and air-dried for 1 h. Sections were then washed with PBS plus Tween 20 (PBST) for 5 min, washed three times in PBS (5 min/wash), incubated with 0.3% hydrogen peroxide in methanol for 30 min to quench endogenous peroxidase activity, blocked by incubation with 5% goat serum (codeX0907, Dako, Denmark) in PBS for 1 h, and then immunostained with a primary antibody against myelin basic protein (1:1000) for 1 h at room temperature to label white matter (including the CC). After three washings with PBS, labeled sections were incubated with secondary antibody (Envision + System-HRP labeled polymer, anti-rabbit, Dako) for 1 h, washed three times with PBS, and treated with Histofine DAB substrate kit (Nichirei Bioscience, Tokyo, Japan) as coloring reagent. The status of the CC was examined under an All-in-One Microscope (BZ-X710; Keyence, Osaka, Japan).

2.7. Statistics

All values are presented as mean ± SEM. Statistical analyses were performed using GraphPad Prism 8 (GraphPad Software, San Diego, CA, USA). Unless otherwise noted, group means were compared by ANOVA with the indicated post hoc test. A $p < 0.05$ (two-tailed) was considered significant for all tests.

3. Results

3.1. Expression of PlxnA1 in the adult mouse brain

To examine the expression of PlxnA1 in the adult (6-month-old) mouse brain, western blotting was performed on protein lysates from several brain regions. As shown in Fig. 1A, PlxnA1 protein was widely expressed in WT mouse brain, especially in the prefrontal cortex, hippocampus, and striatum, but was undetectable in all regions of PlxnA1 KO mouse brain. To visualize the cellular localization of *PlxnA1* mRNA in WT adult mouse brain, in situ hybridization was performed with *PlxnA1* mRNA-specific probes on coronal sections from GAD67-GFP knock-in mice, in which GABAergic neurons are labeled by GFP (Tamamaki et al., 2003; Andrews et al., 2016). Most GFP-positive (GABAergic) neurons in the medial prefrontal cortex and hippocampus of the adult showed detectable PlxnA1 mRNA expression (Fig. 1B, C). Thus, PlxnA1 is expressed in adult as well as developing mouse brain.

3.2. Reduced postnatal survival, body weight, and brain weight of PlxnA1 KO mice

The number of PlxnA1 KO mice born from heterozygous parents was lower than expected by the Mendelian rule at the weaning stage (Total: 192; WT: 59, 30.73%; Heterozygous: 112, 58.33%; KO: 21, 10.94%) but was as expected on postnatal day 0.5 (WT: 22, 26.83%; Heterozygous: 41, 50.00%; KO: 19, 23.17%) (Hossain et al., 2019). Thus, more than half of PlxnA1 KO mice died during the early postnatal period. At 6 months of age, mean body weight of PlxnA1 KO mice was significantly lower than that of WT littermates (25 ± 1.274 g vs. 34.16 ± 0.5911 g, p

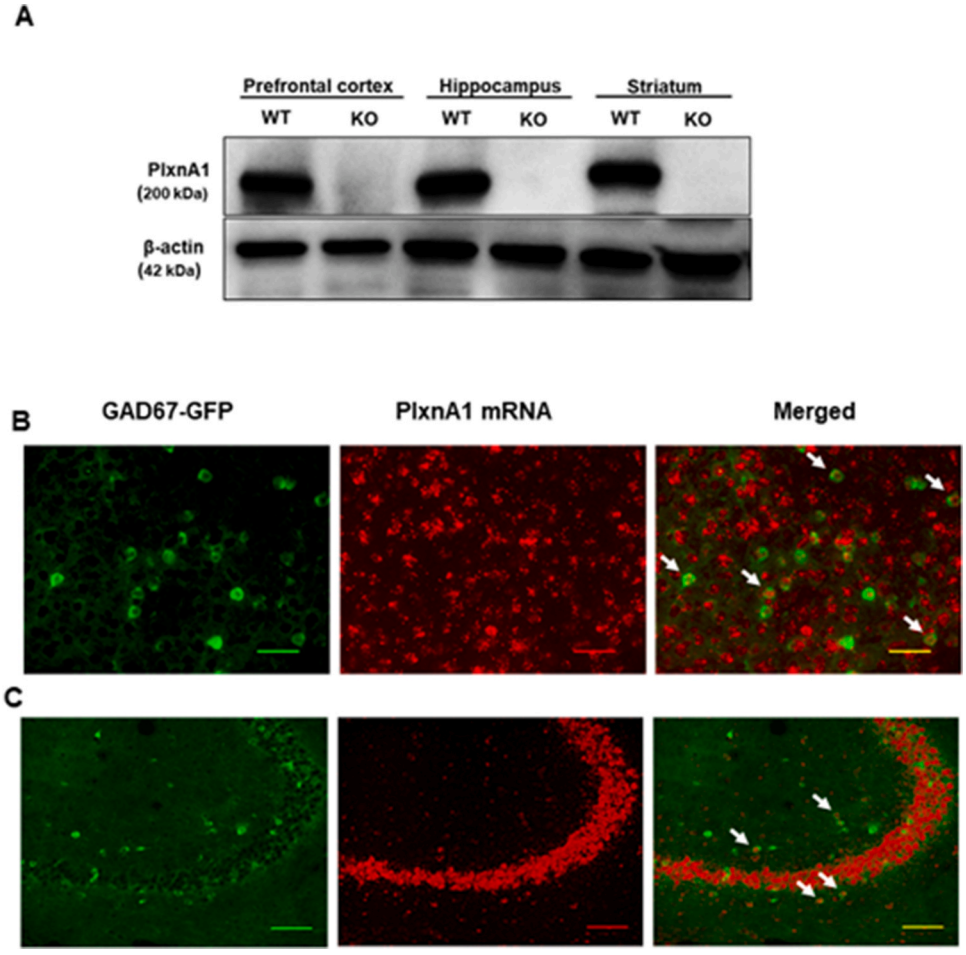


Fig. 1. Expression of PlxnA1 in the adult mouse brain. (A) Western blotting analysis demonstrating the expression of PlxnA1 protein in prefrontal cortex, hippocampus, and striatum of adult WT BALB/cAJ mice (the background for PlxnA1 KO mice). (B) In situ hybridization with a PlxnA1-specific probe detected PlxnA1 mRNA in GFP-expressing GABAergic neurons of the medial prefrontal cortex and hippocampus of GAD67-GFP knock-in mice. WT: wild type, KO: PlxnA1 knockout mice, GFP: green fluorescent protein, GAD67: glutamic acid decarboxylase 67, Arrows: merged cells of GAD67-GFP and PlexnA1 mRNA. Scale bars: 50 μm (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.).

< 0.0001 by Student's *t*-test). Similarly, brain weight at 6 months was significantly lower compared to WT controls (0.4433 ± 0.01 g vs. 0.4756 ± 0.0058 g, p = 0.0132 by Student's *t*-test). Alternatively, neither body nor head weight differed at P0.5 (body weight: 124.00 ± 2.11 mg vs. 125.45 ± 2.52 mg; head weight: 22.79 ± 0.43 mg vs. 22.86 ± 0.49 mg; both p > 0.05 by Student's *t*-test). Thus, PlxnA1 KO mice demonstrate substantial impairment in postnatal body and brain growth.

Agenesis of the corpus callosum (AgCC) was frequently observed in PlxnA1 KO mice at both embryonic day 17.5 (E17.5) and P0.5 (Hossain et al., 2019), but only 13 of 80 adult (6-month-old) PlxnA1 KO mice exhibited AgCC as evidenced by immunohistochemical staining for myelin basic protein (Fig. 2). This finding suggests that these postnatal morphological deficits and early mortality stem, at least in part, from neurodevelopmental abnormalities occurring during embryogenesis.

3.3. Intact limb muscle strength and motor coordination in PlxnA1 KO mice

These developmental abnormalities were reflected by specific behavioral deficits, while other behaviors appeared unaffected despite the aforementioned physical and neuroanatomic abnormalities. For instance, PlxnA1 deficiency had no significant effect on limb muscle strength compared to WTs as assessed by the wire hang test (Fig. 3A; 58.89 ± 1.11 s vs. 58.18 ± 1.82 s, p = 0.7421 by Welch's *t*-test). In the accelerating rotarod test of motor learning, PlxnA1 KO mice showed significantly longer latencies to fall compared to WT mice on all three daily trials (Fig. 3B; main effect of genotype: F (1, 45) = 9.328, p = 0.0038; p = 0.0163 on trial 1, p = 0.0368 on trial 2, p = 0.0334 on trial 3 by Bonferroni's multiple comparisons test).

3.4. Excessive grooming by PlxnA1 KO mice in the open field

The expression of PlxnA1 in GABAergic neurons in prefrontal cortex and hippocampus of adult mice at 6 months suggests the possible role of PlxnA1 in the postnatal brain (Fig. 1). Thus, PlxnA1 deficiency in mice may result in the abnormal behaviors long after maturation. To examine if the absence of PlxnA1 causes the development of abnormal behaviors with age, the open field test was conducted at 2, 4, and 6 months of age. During open field exploration, we measured the effect of genotype and AgCC on total distance traveled, central area entries as a measure of state anxiety, and time spent grooming (Table 1, Fig. 4). There was a significant main effect of genotype (F (1, 42) = 4.33, p = 0.0436) and age (F (2, 84) = 21.76, p < 0.0001) on the total distance traveled but no age × genotype interaction (F (2, 84) = 0.2196, p = 0.8033). Despite the main effect of genotype, there was no significant difference in distance traveled between genotypes at 2, 4, and 6 months (Table 1, all p > 0.05 by Bonferroni test; Supplementary Fig. 1A). To examine the effect of AgCC on total distance traveled (Supplementary Fig. 1B), PlxnA1 KO mice were retrospectively divided into those with an intact CC (PlxnA1 KO-CC group) and those with substantial AgCC (PlxnA1 KO-AgCC group). While there was a significant main effect of age (Table 1, F (2,82) = 17.82, p < 0.0001), there was no main effect of group (Table 1, PlxnA1 KO-CC, PlxnA1 KO-AgCC, and WT; F (2, 41) = 2.282, p = 0.1149) or age × group interaction (F (4, 82) = 0.3762, p = 0.8250). Two-way ANOVA revealed a significant main effect of genotype on both the number of entries into the central zone (Supplementary Fig. 1C; F (1, 43) = 7.100, p = 0.0108) and time spent in the central zone (Supplementary Fig. 1E; F (1, 43) = 4.505, p = 0.0396), and 4-month-old PlxnA1 KO mice entered the central zone significantly more frequently than age-matched WTs (Table 1, Supplementary Fig. 1C; p < 0.05 by

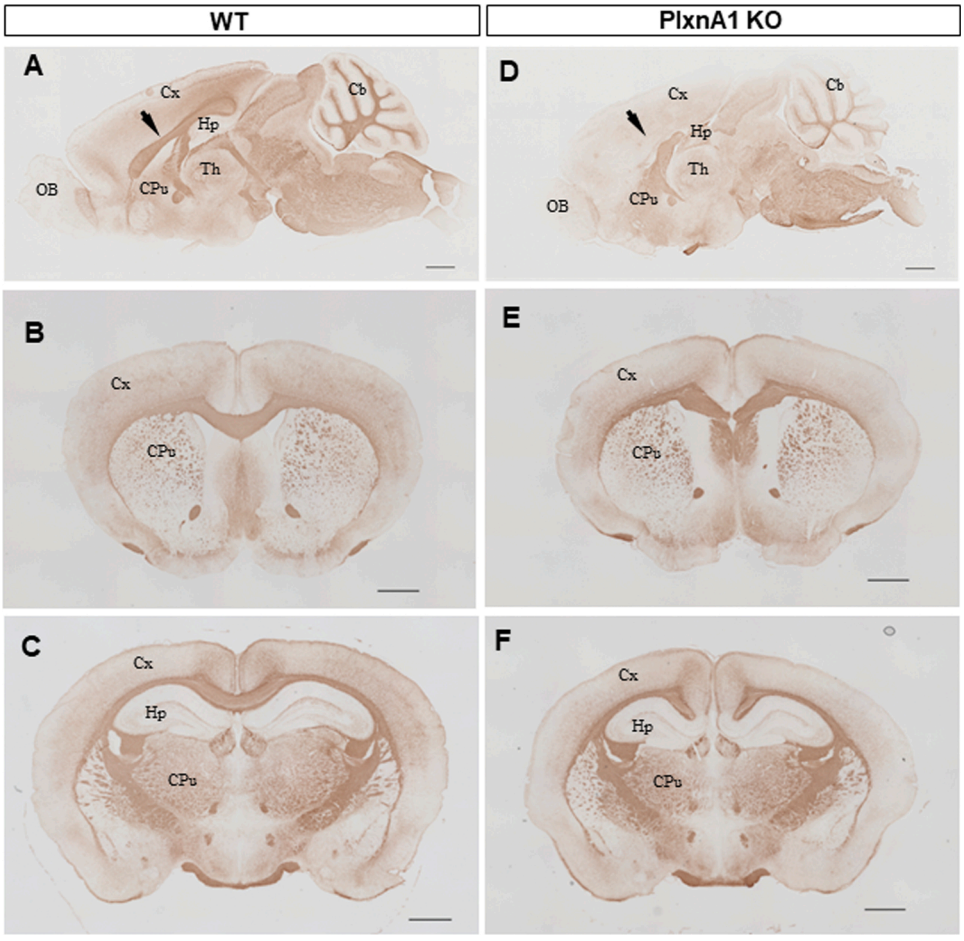


Fig. 2. Comparison of corpus callosum formation in WT and PlxnA1 KO mice. (A-C) The corpus callosum (CC) was detected by immunostaining for myelin basic protein (brown color) at mid-sagittal plane of WT brain (arrow in A), and at coronal planes in both the rostral (B) and caudal brain (C) of WT mice. (D-F) The CC was not detected at the mid-sagittal plan (arrow in D), rostral coronal plan (E), or caudal coronal plan (F) in 5 of 21 PlxnA1 KO mice (termed PlxnA1 KO-AgCC mice). WT (n = 23); PlxnA1 KO (n = 21); PlxnA1 KO-AgCC (n = 5). WT: wild type, KO: PlxnA1 knockout mice, PlxnA1 KO-AgCC: PlxnA1 KO mice with agenesis of the corpus callosum. OB: olfactory bulb, Cx: cortex, Hp: hippocampus, Th: thalamus, CPu: caudate putamen, Cb: cerebellum. Scale bars: 1000 μm.

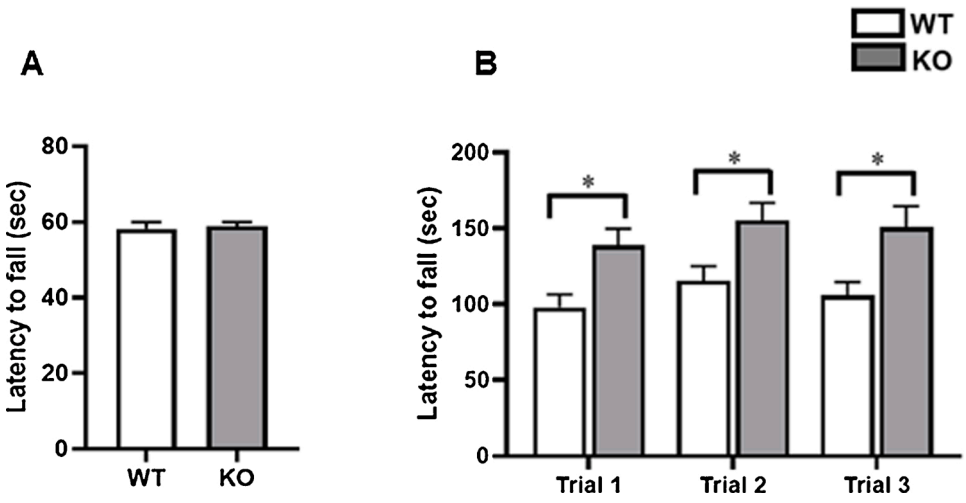


Fig. 3. Limb strength and motor coordination were not deficient in PlxnA1 KO mice. (A) No difference in wire hang time was observed between PlxnA1 KO and WT mice (A). WT (n = 22); PlxnA1 KO (n = 18). (B) PlxnA1 KO mice exhibited significantly longer latencies to fall during the rotarod test than WT mice. WT (n = 32); PlxnA1 KO (n = 15), Data are presented as mean ± SEM. WT: wild type, KO: PlxnA1-deficint mice.

Table 1
Behavioral phenotypes of PlxnA1 KO mice in the open field test.

Behavioral phenotype	2 months			4 months			6 months		
	KO	KO-CC	KO-AgCC	KO	KO-CC	KO-AgCC	KO	KO-CC	KO-AgCC
Total distance	NS	NS	NS	NS	NS	NS	NS	NS	NS
Central zone (number)	NS	↑	NS	↑	↑	NS	NS	NS	NS
Central zone (time)	NS	NS	NS	NS	NS	NS	NS	NS	NS
Grooming (number)	NS	NS	NS	NS	NS	NS	↑↑↑↑	↑	↑↑*
Grooming (time)	NS	NS	↑	NS	NS	NS	↑↑↑↑	↑	↑↑↑↑*

NS, not significant; ↑, ↑↑, ↑↑↑↑: significantly increased compared with WT, ↑: $p < 0.05$, ↑↑: $p < 0.01$, ↑↑↑↑: $p < 0.0001$, ↑↑*, ↑↑↑↑* : significantly increased also in comparison with KO-CC in addition to the comparison with WT.
KO, PlxnA1 KO mice; KO-CC, PlxnA1 KO mice with an intact corpus callosum (CC); KO-AgCC, PlxnA1 KO mice with substantial agenesis of corpus callosum (AgCC).

Bonferroni test). In contrast, there were no significant genotype differences in central zone time at any age (Table 1, all $p > 0.05$ by Bonferroni test). There were significant main effects of AgCC group and age on the number of entries into the central zone (Supplementary Fig. 1D; $F(2, 42) = 4.476$, $p = 0.0173$; $F(2, 84) = 9.300$, $p = 0.0002$). At 2 and 4 months of age, PlxnA1 KO-CC mice made significantly more entries into the central zone than age-matched WTs (Table 1, Supplementary Fig. 1D; $p < 0.05$ by Bonferroni test). There was also a significant main effect of AgCC group on time spent in the central zone (Supplementary Fig. 1F; $F(2, 42) = 3.605$, $p = 0.0359$), but PlxnA1 KO-AgCC and PlxnA1 KO-CC mice did not differ significantly in number of entries into the central zone or time spent in the central zone at any age (Table 1, Supplementary Fig. 1D and 1 F; both $p > 0.05$ by Bonferroni test).

However, PlxnA1 KO did exhibit significantly enhanced self-grooming behavior in the open field. There were significant main effects of age (Fig. 4A; $F(2, 86) = 7.868$, $p = 0.0007$) and genotype (Fig. 4A; $F(1, 43) = 9.953$, $p = 0.0029$) on number of grooming episodes in the open field as well as a significant age × genotype interaction ($F(2, 86) = 5.097$, $p = 0.0081$). Moreover, Bonferroni's multiple comparison tests revealed a significant increase in the number of grooming episodes by PlxnA1 KO mice compared to WTs at 6 months of age (Table 1, Fig. 4A; $p < 0.0001$). There was also a significant main effect of age (Fig. 4C; $F(2, 86) = 6.288$, $p = 0.0028$) and genotype (Fig. 4C; $F(1, 43) = 11.17$, $p = 0.0017$) on the duration of grooming in the open field test but no age × genotype interaction ($F(2, 86) = 2.399$ $p = 0.0969$). Bonferroni's multiple comparison tests confirmed a significant increase in grooming duration by PlxnA1 KO mice compared to WT mice at 6 months old (Table 1, Fig. 4C; $p < 0.0004$). Finally, there was a significant main effect of age (Fig. 4B; $F(2, 84) = 18.38$, $p < 0.0001$) and AgCC group (Fig. 5B; $F(2, 42) = 6.412$, $p = 0.0037$), and a significant age × group interaction ($F(4, 84) = 6.241$, $p = 0.0002$) on number of grooming episodes, with PlxnA1 KO-AgCC mice showing a significantly greater number than WT and PlxnA1 KO-CC mice at 6 months of age (Table 1, Fig. 4B; $p < 0.0001$ vs. WT and $p = 0.0017$ vs. PlxnA1 KO-CC by Bonferroni test). Also at 6 months of age, PlxnA1 KO-CC mice demonstrated a significantly greater number of grooming episodes compared to WT mice (Table 1, Fig. 4B; $p = 0.0122$ by Bonferroni test). In addition, there was a significant main effect of age (Fig. 4D; $F(2, 94) = 11.08$, $p < 0.0001$) and AgCC group (Fig. 4D; $F(2, 47) = 11.99$, $p < 0.0001$) on grooming duration and a significant age × group interaction ($F(4, 94) = 3.201$, $p = 0.0164$). At 2 and 6 months of age, grooming duration was significantly longer among PlxnA1 KO-AgCC mice compared to WT mice (Table 1, Fig. 4D; $p = 0.0210$ at 2 months and $p < 0.0001$ at 6 months by Bonferroni test). At 6 months of age, PlxnA1 KO-AgCC mice demonstrated significantly longer grooming times than PlxnA1 KO-CC mice (Table 1, Fig. 4D; $p = 0.0026$ by Bonferroni test), while PlxnA1 KO-CC mice exhibited significantly longer grooming times than WT mice (Table 1, Fig. 4D; $p = 0.0430$ by Bonferroni test). Thus, PlxnA1 KO appears to enhance self-grooming behavior, and this effect is exacerbated by CC agenesis.

3.5. *Grooming behavior in the home cage*

As the open field environment may induce stress and thus alter a broad range of spontaneous behaviors, we also examined grooming in the home cage during the active (dark) phase from 20:00 to 8:00 using another cohort of WT and PlxnA1 KO mice. Consistent with findings in the open field, there was a significant main effect of genotype (Fig. 5A; F

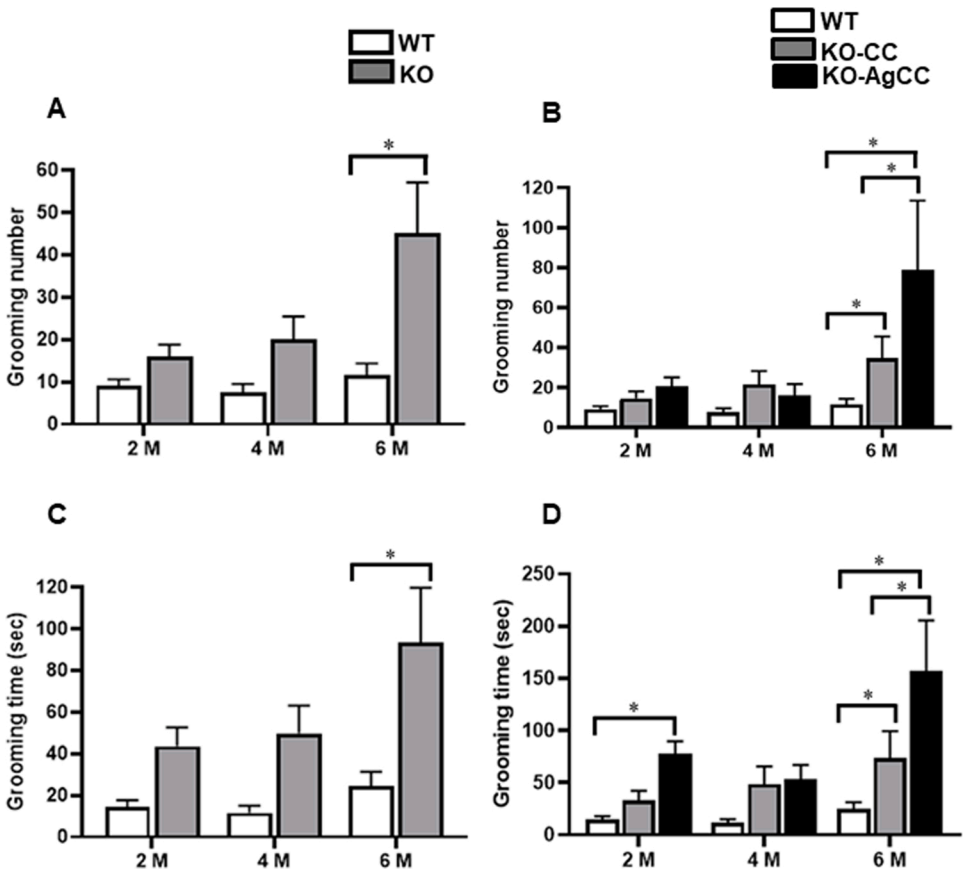


Fig. 4. Excessive grooming behavior by PlxnA1 KO mice in the open field. (A) PlxnA1 KO mice at 6 months old exhibited a significantly greater number of grooming episodes compared to age-matched WT mice. (B) PlxnA1 KO-CC at 6 months old exhibited significantly longer grooming episodes than age-matched WT mice. (C) PlxnA1 KO-AgCC at 6 months of age showed significantly higher numbers of grooming episodes compared to age-matched PlxnA1 KO-CC and WT mice. PlxnA1 KO mice at 6-month old exhibited significantly longer grooming times than age-matched WT mice. (D) PlxnA1 KO-CC mice at 6 months exhibited significantly longer grooming times than age-matched WT mice. PlxnA1 KO-AgCC mice also exhibited significantly longer grooming times at 2 and 6 months old compared to age-matched WT mice. WT (n = 23); PlxnA1 KO (n = 21); PlxnA1 KO-CC (n = 16); PlxnA1 KO-AgCC (n = 5). Data are presented as mean ± SEM. WT: wild type, KO: PlxnA1 knockout mice, PlxnA1 KO-CC: PlxnA1 KO mice with corpus callosum, PlxnA1 KO-AgCC: PlxnA1 KO mice with agenesis of corpus callosum. 2M: 2 months old, 4M: 4 months old, 6M: 6 months old.

(1, 43) = 10.87, p = 0.0020) on grooming time as well as a main effect of time bin (hour) within the observation period (Fig. 5A; F (5, 215) = 33.89, p < 0.0001) and a significant genotype × hour interaction (F (5, 215) = 4.598, p = 0.0005). PlxnA1 KO mice demonstrated significant longer grooming times during the 20:00–22:00 period than WT mice (Fig. 5A; p < 0.0001 by Bonferroni test). There were also significant main effects of genotype (Fig. 5B; F (1, 43) = 286.6, p < 0.0001) and hour (Fig. 5B; F (5, 215) = 13.10, p < 0.0001) on scratching time and a significant genotype × hour interaction (F (5, 215) = 7.426, p < 0.0001). Bonferroni's multiple comparison tests confirmed a significant increase in scratching time by PlxnA1 KO mice during all time bins compared to WT mice (Fig. 5B; all p < 0.0001).

3.6. Decreased prepulse inhibition in PlxnA1 KO mice

The baseline acoustic startle response did not differ between genotypes (Fig. 6A; p > 0.05 by Welch's *t*-test) but there was a significant main effect of genotype (Fig. 6B; F (1, 62) = 5.688, p = 0.0202) and prepulse condition (Fig. 6B; F (2, 124) = 56.47, p < 0.0001) on %PPI, as well as a significant genotype × prepulse interaction (F (2, 124) = 3.900, p = 0.0228). Further, PlxnA1 KO mice demonstrated a significant decrease in %PPI at a prepulse intensity of 86 dB compared to WT mice (Fig. 6B; p = 0.0068 by Bonferroni test). There was also a significant main effect of AgCC group (Fig. 6C; F (2, 61) = 3.451, p = 0.0380) and prepulse intensity (Fig. 6C; F (2,122) = 20.40, p < 0.0001) but no significant group × prepulse intensity interaction (F (4, 122) = 1.964, p = 0.1042). PlxnA1 KO-CC mice demonstrated significantly reduced %PPI at both 78 dB and 86 dB prepulse intensities compared to WT mice (Fig. 6C; 78 dB: p = 0.0310 by Bonferroni test, 86 dB: p = 0.0050 by Bonferroni test), while there were no significant differences between WT and PlxnA1 KO-AgCC mice or between PlxnA1 KO-CC and PlxnA1 KO-AgCC mice for any test condition (Fig. 6C).

3.7. Reduced anxiety-like behavior of PlxnA1 KO mice in the elevated plus maze test

In the elevated plus maze test, PlxnA1 KO mice spent significantly more time on the open arms than WT mice (Fig. 7B; 197.3 ± 32.27 vs. 85.30 ± 21.20 s, p = 0.0096 by Student's *t*-test) and significantly less time on the closed arms (Fig. 7E; 99.95 ± 33.61 vs. 206.8 ± 22.71 s, p = 0.0140 by Student's *t*-test), suggesting that PlxnA1 KO reduces anxiety-like behavior (consistent with more numerous central zone entries in the open field).

3.8. Social interaction of PlxnA1 KO mice is not different from the WT mice

Both WT and PlxnA1 KO mice showed a significant preference for exploring the cage housing stranger mouse 1 compared to the empty cage (Fig. 8A; F (3, 52) = 5.345, p = 0.0028; WT: empty vs. stranger 1, p = 0.0473; KO: empty versus stranger 1, p = 0.0335). In the following trail with the original stranger 1 and a novel mouse (stranger 2) in separate cages, the number of interactions and time of close contact visitations did not differ between WT and PlxnA1 KO mice (Fig. 8C; F (3, 52) = 2.825, p = 0.0476; WT: stranger 1 vs. stranger 2, p = 0.1880; KO: stranger 1 vs. stranger 2, p > 0.9999). Thus, PlxnA1 KO did not alter social behavior.

3.9. Normal working and fear memory in PlxnA1 KO mice

The Y maze spontaneous alternation test did not reveal significant differences in working memory between PlxnA1 KO mice and WT controls (Fig. 9A; 56.77% ± 3.91% vs. 64.87% ± 2.85%, p > 0.05 by Student's *t*-test). However, PlxnA1 KO mice made significantly fewer total arm entries compared to WT mice (Fig. 9B; 20.07 ± 1.96 vs. 26.28 ±

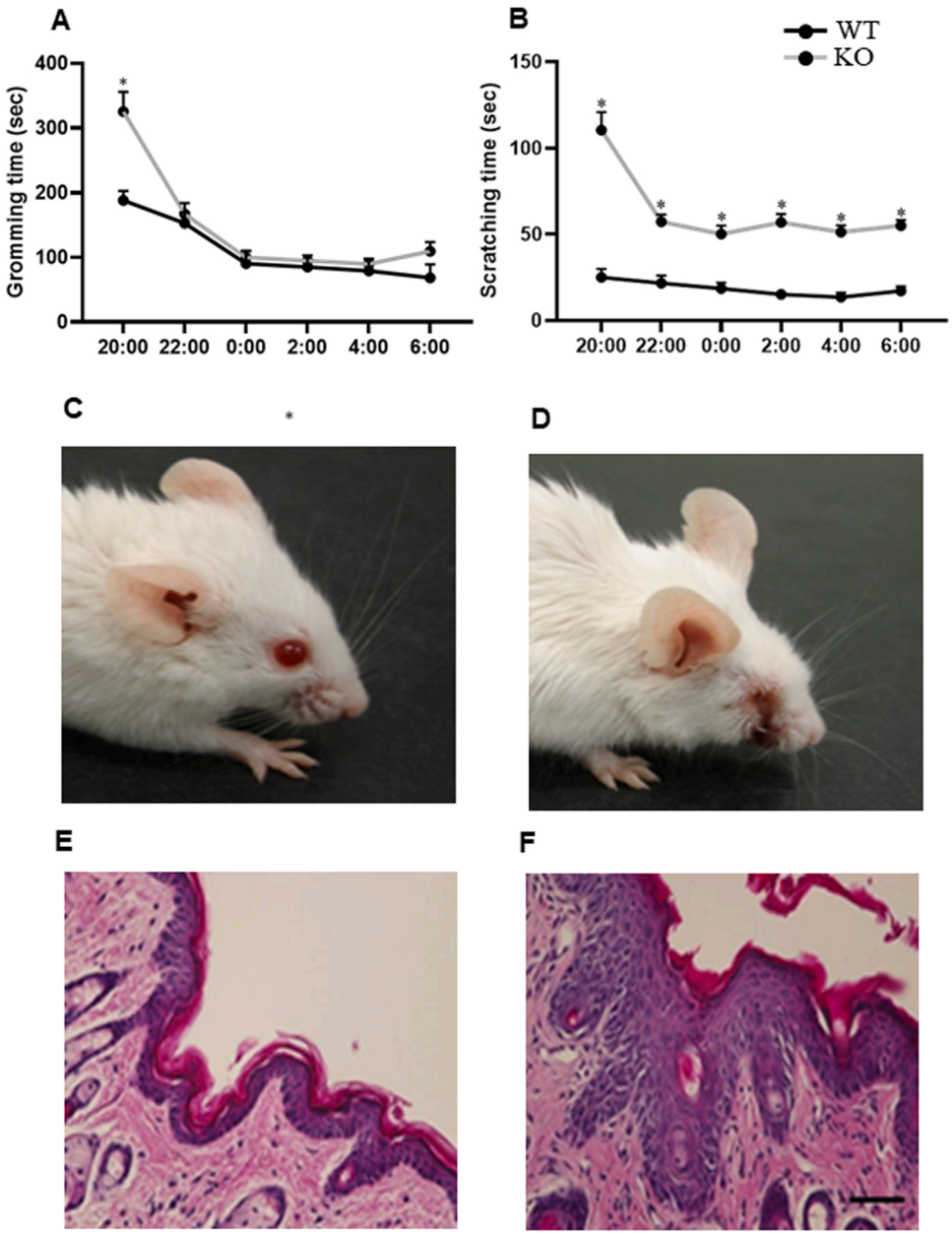


Fig. 5. Excessive grooming behavior of PlxnA1 KO mice in the home cage. (A, B) PlxnA1 KO mice demonstrated significantly greater grooming behavior during the period from 20:00 to 22:00 and greater scratching behavior (B) during all time bins compared to WT mice. WT (n = 20); PlxnA1 KO (n = 25). Data are presented as mean ± SEM. WT: wild type, KO: knockout: PlxnA1-deficint mice. (C, D) Severe skin lesions around the eyes, presumably caused by excessive grooming, were observed in several PlxnA1 KO mice (D) but absent in WT mice (C). (E, F) Hematoxylin-eosin staining confirmed increased thickness of the epidermis under the eyes of PlxnA1 KO mice (F) but not WT mice (E). WT: wild type, KO: PlxnA1 knockout mice.

1.49, $p < 0.05$ by Student's *t*-test), indicating lower exploratory activity. Similarly, there were no differences in freezing time (fear memory) between PlxnA1 KO and WT mice under either the contextual condition (Fig. 9C 12.54 ± 2.896 s vs. 11.40 ± 4.200 s, $p > 0.05$ by Student's *t*-test) or the cued condition (Fig. 9D; 26.18 ± 4.69 s vs. 25.63 ± 4.410 s, $p > 0.05$ by Student's *t*-test). In summary, these findings indicate that PlxnA1 KO had no effects on working memory, long-term contextual fear memory, or long-term cued fear memory.

4. Discussion

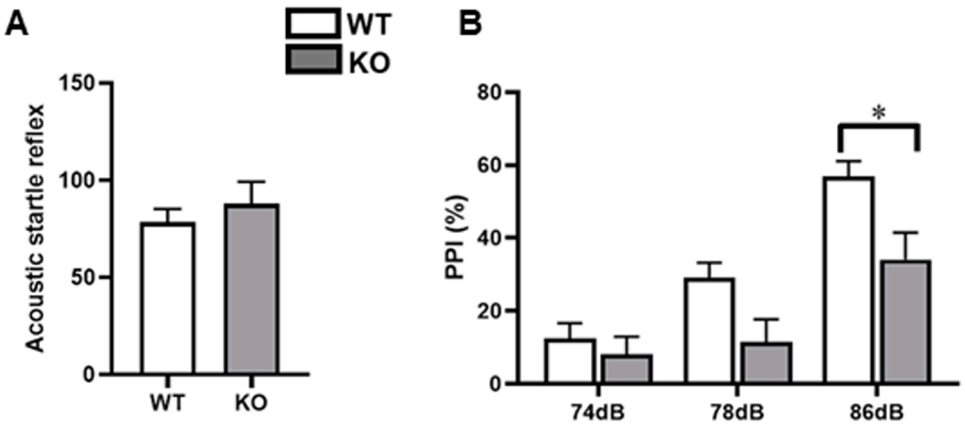
4.1. Expression of PlxnA1 in adult mouse brain

The present study confirmed PlxnA1 mRNA expression by GABAergic interneurons in both prefrontal cortex and hippocampus of adult mice. PlxnA1 KO mice demonstrated poor postnatal survival until weaning and impaired body and brain growth thereafter. Further, behavioral tests revealed multiple abnormalities in adulthood, including excessive self-grooming in both the open field and home cage as well as reduced PPI.

PlxnA1 mRNA expression by adult BALB/cAJ mice was enriched in GABAergic neurons of both prefrontal cortex and hippocampus (Fig. 1B, C), consistent with previous studies reporting immunohistochemical detection of PlxnA1 in developing GABAergic neurons of embryonic C57BL/6 J mouse brain (Andrews et al., 2016) and parvalbumin-positive GABAergic neurons of adult mouse brain (Vo et al., 2013).

4.2. Decreased postnatal survival, body weight and brain weight

At P0.5, PlxnA1 KO mice were observed at the expected Mendelian ratio of ~25 % and neither body nor head weight differed significantly from WT littermates. Thus, PlxnA1 deficiency did not have a lethal effect during BALB/cAJ mouse embryogenesis, but more than half died from P0.5 to weaning by an unknown cause. One possible causes of the postnatal death may be AgCC observed at high frequency in PlxnA1 KO mice at P0.5 (Hossain et al., 2019). One possible explanation is seizure activity, as *PlxnA1* allelic variants are associated with intractable



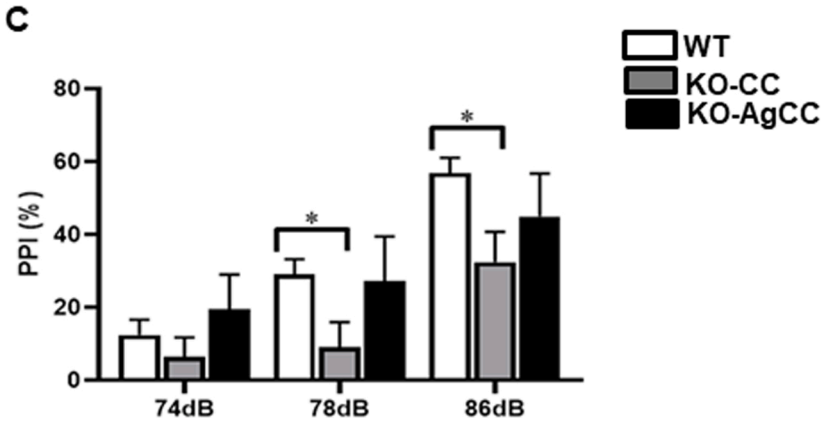


Fig. 6. Impaired prepulse inhibition (PPI) in PlxnA1 KO mice. (A) The amplitude of the auditory startle reflex did not differ between PlxnA1 KO and WT mice. (B) %PPI was significantly reduced in PlxnA1 KO mice at a prepulse intensity of 86 dB compared to WT mice. (C) PlxnA1 KO-CC mice displayed significantly reduced %PPI at both 78 dB and 86 dB prepulse intensities compared to WT mice (C). WT (n = 34); PlxnA1 KO (n = 30); PlxnA1 KO-CC (n = 26); PlxnA1 KO-AgCC (n = 4). Data are presented as mean ± SEM. WT: wild type, KO: PlxnA1 knockout mice, PlxnA1 KO-CC: PlxnA1 KO mice with corpus callosum, PlxnA1 KO-AgCC: PlxnA1 KO mice with agenesis of corpus callosum.

epilepsy in humans (Epi4K Consortium et al., 2013; Fromer et al., 2014; Oliver et al., 2016; Park et al., 2017). However, lethal complications like epilepsy have not been observed in adult PlxnA1 KO mice, even those with AgCC. Alternatively, AgCC or interneuronal abnormalities may affect maternal bonding or suckling. Additional studies investigating the nutritional status of these mice will be needed to reveal the cause of early postnatal death.

4.3. Unimpaired motor function and hyper-grooming of PlxnA1 KO mice

Despite impaired postnatal growth, PlxnA1 KO mice displayed normal limb muscle strength in the wire hang test (Fig. 3A). Furthermore, PlxnA1 KO mice actually demonstrated superior motor coordination in the rotarod test (Fig. 3B). Though lower body weight alone may facilitate rotarod performance, it is also possible that PlxnA1 KO improves limb coordination. Conditional PlxnA1 KO specific to *Emx1*-expressing excitatory neurons resulted in superior manual dexterity compared to WT mice by maintaining stronger cortico–motoneuronal connections into adulthood (Gu et al., 2017). We speculate that constitutive PlxnA1 KO mice may switch hands more skillfully during rod rotation due to superior manual dexterity, although this remains to be examined.

While most behavioral metrics did not differ from WT littermates, PlxnA1 KO mice (Figs. 4 and 5) exhibited significant hyper-grooming behavior in both the open field and the home cage. Hyper-grooming is often regarded as a sign of anxiety in mice (Smolinsky et al., 2009). However, theses mice made more frequent entries into the central zone of the open field than WTs (Table 1, Supplementary Fig. 1C, 1D), behaviors associated with reduced anxiety in mice (Puschban et al., 2016). Furthermore, PlxnA1 KO mice spent significantly more time on the open arms and significantly less time on the closed arms of an elevated plus maze (Fig. 7), again indicative of reduced anxiety (Berkowicz et al., 2016). Thus, the observed hyper-grooming behavior is unlikely attributable to greater trait anxiety.

The excessive self-grooming in the home cage was associated with skin lesions around the eyes (Fig. 5), indicative of its severity. Similar excessive self-grooming in the home cage was reported in mice with a deficiency in the PlxnA coreceptor neuropilin 2 (Shiflett et al., 2015; Singer, 2013; Muehlmann and Lewis, 2012). Excessive self-grooming in rodents is considered a valuable endophenotype for pathological conditions characterized by repetitive behaviors (Berkowicz et al., 2016; Kalueff et al., 2016). However, these PlxnA1 KO mice exhibited a behavioral profile distinct from other such models. For instance, both Sema5A KO mice (Duan et al., 2014) and interneuron-specific Sema3 F KO mice (Li et al., 2019) exhibited social deficits but no hyper-grooming, while our PlxnA1 KO mice did not show social deficits (Fig. 8). Repetitive behaviors including hyper-grooming have been observed in several autism spectrum disorder (ASD) models (Jamain et al., 2008; Yang et al., 2012). Therefore, the mechanisms underlying hyper-grooming in PlxnA1 KO mice may still be relevant to neurodevelopmental disorders characterized by stereotypies.

The open-field test revealed the significant increase of grooming in PlxnA1 KO mice at 6 months of age, and thus the hyper-grooming in PlxnA1 KO mice developed along with age (Fig. 4A, C). The mice deficient of the SAP90/PSD-95 associated protein (Sapap3; DLGAP3; GKAP3) shows the similar age-dependent increase in the duration of

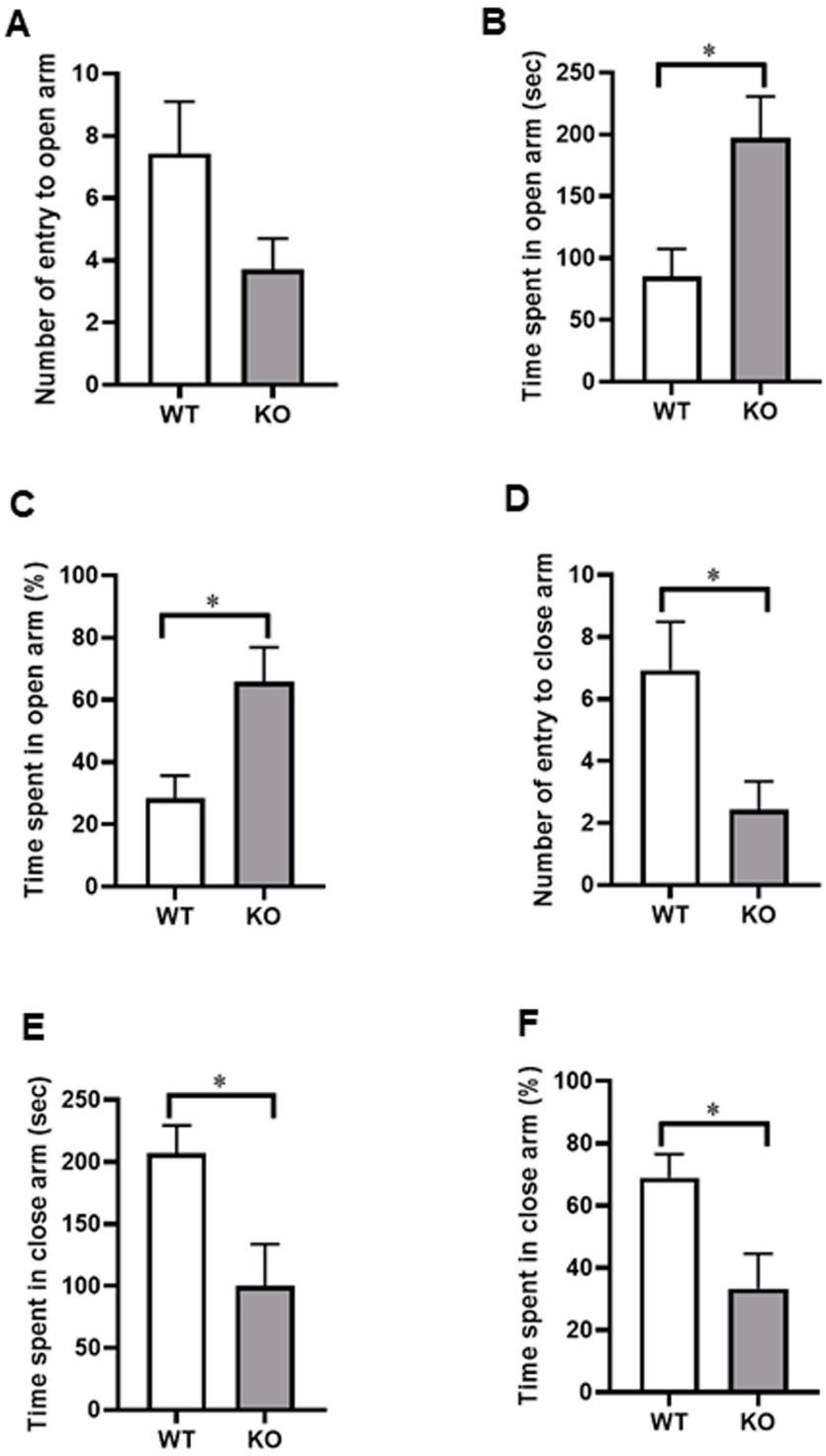


Fig. 7. Reduced anxiety-like behavior by PlxnA1 KO mice in the elevated plus maze. (A-E) PlxnA1 KO mice spent significantly more time on the open arms (B), a significantly higher percentage (%) of time on the open arms (C), significantly fewer closed arm entries (D), and significantly less time on the closed arms than WT mice (E). (F) KO mice also made a significantly lower percentage (%) of time on the closed arms than WT mice. WT (n = 14); PlxnA1 KO (n = 14). Data are presented as mean ± SEM. WT: wild type, KO: PlxnA1 knockout mice.

grooming (Glorie et al., 2020). The age-dependent increase in the duration of grooming in Sapap3 KO mice is indicated to associate with an age-dependent decline in mGluR5 availability in the cortex, striatum, hippocampus, and amygdala which are relevant to obsessive compulsive disorder (Glorie et al., 2020). Thus, the effect of ligands for PlxnA1 in PlxnA1 KO mice at young age may be preserved in brain areas relevant to the control of grooming behavior by the compensatory function of PlxnA family members (PlxnA2, A3, A4). However, the effect of ligands for PlxnA1 may be weakened in PlxnA1 KO mice with advancing age due to the decompensation of PlxnA1 function, and significant increase of grooming may be evident in PlxnA1 KO mice at 6 months of age.

The agenesis of corpus callosum (AgCC) significantly enhanced the degree of self-grooming behavior in PlxnA1 KO mice at 6 months of age (Fig. 4 B, D). Significantly increased self-grooming behaviors are also observed in BTBR T+tf/J (BTBR) strain of mice along with a complete congenital AgCC (Chao et al., 2018), consistent with other reports (Moy et al., 2007; Yang et al., 2007; McFarlane et al., 2008). However, subtle disruptions of neurodevelopment other than AgCC are indicated to contribute to low sociability and repetitive self-grooming in BTBR strain of mice (Yang et al., 2009). Since PlxnA1 KO mice with an intact CC (PlxnA1 KO-CC) showed a significant increase of grooming in contrast to WT at 6 months (Fig. 4B and D), other abnormalities other than CC may

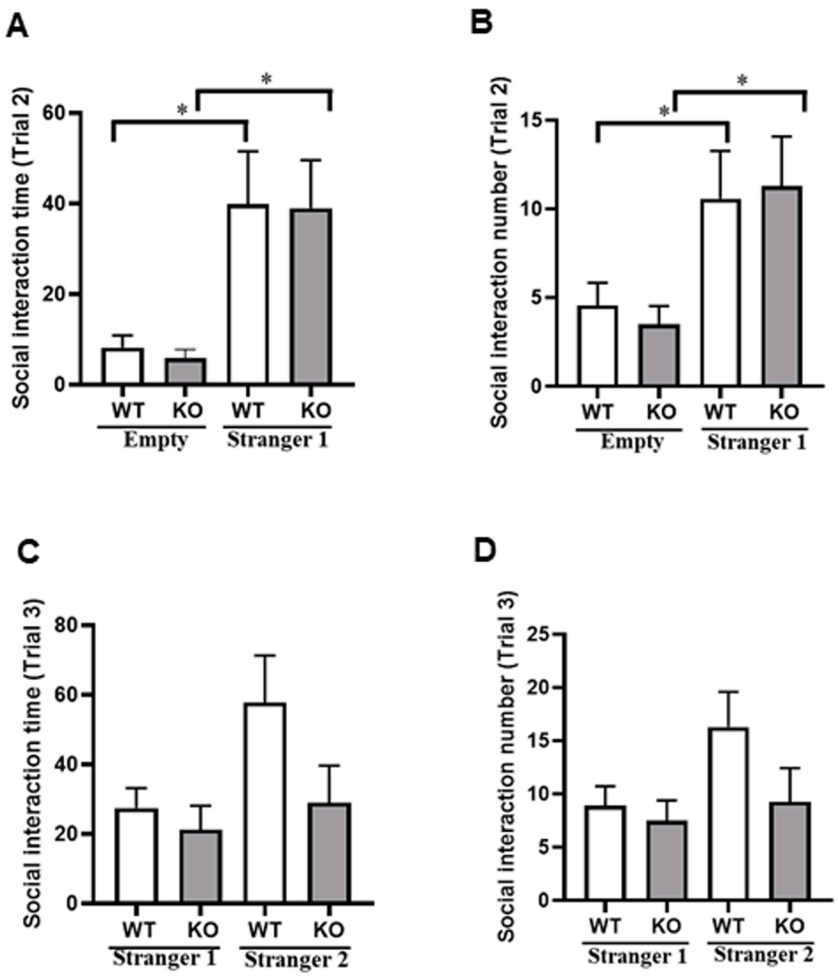


Fig. 8. Normal social behavior of PlxnA1 KO mice. (A, B) Both WT and PlxnA1 KO mice demonstrated a significant preference for the cage containing a stranger mouse (1) compared to an empty cage. (C, D) Neither genotype demonstrated a significant preference for the cage containing a novel mouse (stranger 2) compared to stranger 1. WT (n = 14); PlxnA1 KO (n = 14). Data are presented as mean ± SEM. WT: wild type, KO: PlxnA1 knockout mice.

underlie the increased grooming in PlxnA1 KO mice. Thus, in PlxnA1 KO mice, AgCC may augment the hyper-grooming caused by dysfunction of neural circuitry or subtle neurodevelopmental abnormalities other than AgCC.

4.4. *PPI deficits in PlxnA1 KO mice*

These PlxnA1 KO mice also exhibited significant PPI impairment, an endophenotype of the sensorimotor gating dysfunction observed in schizophrenia patients (Braff et al., 1992; Mena et al., 2016; Takao et al., 2007; Kumari et al., 2008; Yang et al., 2017). Among mice harboring mutations of PlxnA family members, both constitutive PlxnA2 KO mice and PlxnA2-GAP-deficient mice exhibit PPI deficits (Zhao et al., 2018). Further, allelic variants of human *PlxnA2* are associated with schizophrenia (Mah et al., 2006). We speculate that PlxnA1–Sema signaling may also contribute to the development of neural circuitry required for PPI.

As to the relationship between PPI and AgCC, BTBR mice with AgCC shows normal PPI (Silverman et al., 2010b). Thus, AgCC itself may not be responsible for the impairment of PPI. Since PlxnA1 KO-CC (PlxnA1 KO mice with an intact CC) showed the significant decrease of PPI and PlxnA1 KO-AgCC (PlxnA1 KO mice with substantial AgCC) did not exhibit the significant differences in PPI as compared with either WT or KO-CC (Fig. 6C), contribution of AgCC to the PPI deficit may be low in PlxnA1 KO mice.

4.5. *PlxnA1 KO mice recapitulate only limited features of developmental encephalopathy and schizophrenia*

Three humans with distinct missense mutations of *PlxnA1* demonstrated developmental encephalopathy or schizophrenic symptoms (Epi4K Consortium et al., 2013; Fromer et al., 2014; Oliver et al., 2016; Park et al., 2017). Thus, it is of interest to evaluate the extent to which the neurobehavioral phenotypes of PlxnA1 KO mice overlap with the symptoms of these diseases. Developmental encephalopathies comprise an extensive and genetically heterogeneous range of diseases associated with global developmental delay, intellectual disability, frequent epilepsy, and other neurofunctional impairments (Park et al., 2017; Kalser and Cross, 2018; Hebbar and Mefford, 2020). A patient with a heterozygous missense mutation of *PlxnA1* showed developmental encephalopathy characterized by intractable infantile-onset epilepsy and intellectual disability with ASD. The patient also displayed features suggestive of Dubowitz syndrome (growth failure, cutaneous symptoms, and dysmorphic facial features) (Park et al., 2017). While PlxnA1 KO mice exhibited body and brain growth impairments, we did not detect seizure activity, abnormal sociability, impaired working memory, or deficient long-term memory.

Nonetheless, the substantial behavioral and information processing deficits displayed by PlxnA1 KO mice may provide a valuable model for mechanistic investigations. Prepulse inhibition is regarded as a useful, reliable, robust, and quantitative phenotype for exploring the neurobiology and genetics of gating disorder in schizophrenia (Swerdlow et al.,

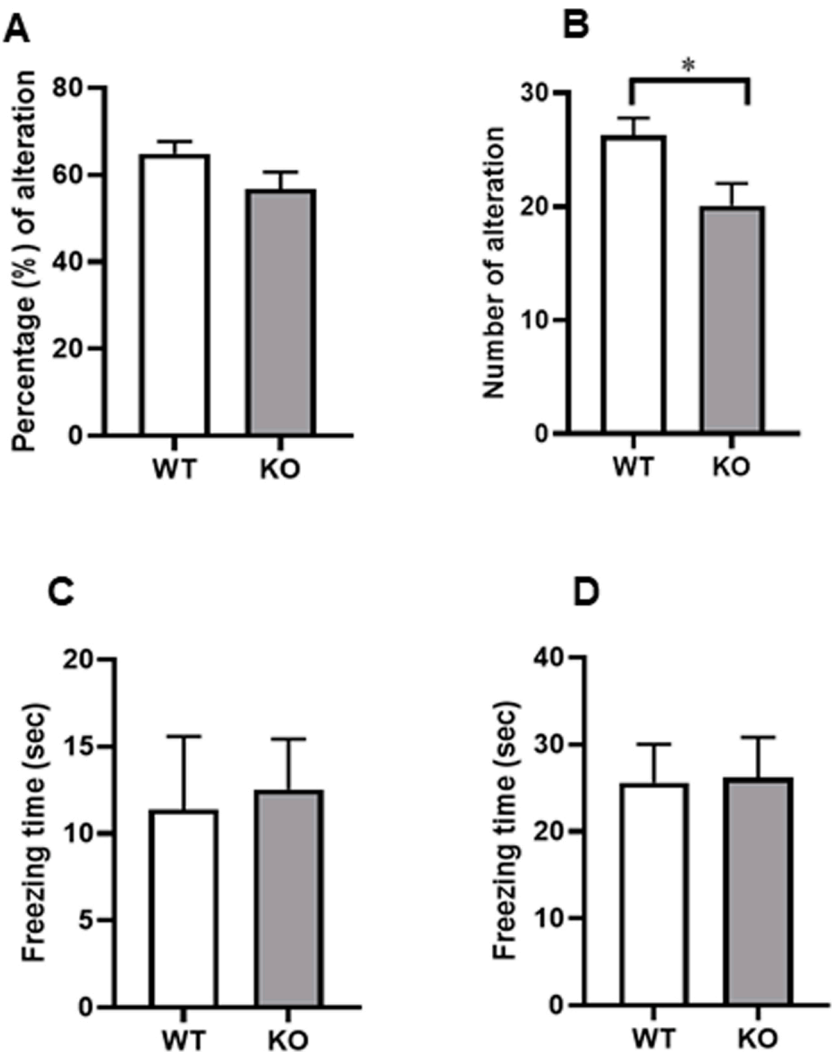


Fig. 9. Normal working and fear memory in PlxnA1 K O mice. (A, B) Percentage alternations in the Y maze test (A) and number of arm entries (B) by WT and PlxnA1 KO mice. (C, D) No significant difference in freezing time by PlxnA1 KO mice compared to WT mice in both the contextual fear conditioning test (C) and cued fear conditioning test (D). WT (n = 14); PlxnA1 KO (n = 14). Data are presented as mean ± SEM. WT: wild type, KO: PlxnA1 knockout mice.

2008). Recent methylation and gene ontology enrichment analyses have identified several biological processes with potential relevance to schizophrenia pathophysiology (Neary et al., 2017). Hyperlocomotor activity in mice is also recognized as an endophenotype of mania and positive symptoms of schizophrenia (Van den Buuse, 2010), but this was not observed in PlxnA1 KO mice (Table 1, Supplementary Fig. 1A and 1B).

Since PlxnA1 KO mice displayed reduced anxiety in the open field and elevated plus maze test, hyper-grooming appears unrelated to anxiety. Reduced anxiety and normal sociability of PlxnA1 KO mice also suggest that hyper-grooming is not associated with pathomechanisms relevant to ASD or obsessive-compulsive disorder. Alternatively, stereotypical behavior like hyper-grooming has been observed as a positive symptom in rodent models of schizophrenia (Onaolapo et al., 2017; Dias et al., 2019). Thus, increased self-grooming and excessive scratching behavior in PlxnA1 KO mice may be related to the stereotypical behaviors characteristic of schizophrenia, although PlxnA1 KO mice did not display other schizotypic phenotypes such as impaired sociability (Fig. 8) and memory (Fig. 9). Taken together, PlxnA1 KO mice appear to exhibit two specific features relevant to the pathophysiology of schizophrenia. PlxnA1 KO mice may thus be a unique mouse model to explore the mechanisms of stereotypical behaviors and impaired PPI underlying neurodevelopmental or psychiatric disorders.

5. Conclusion

PlxnA1 KO mice demonstrated poor survival during the early postnatal period as well as impaired body and brain growth into adulthood. Behavioral testing revealed excessive self-grooming in both the open field and home cage as well as impaired PPI. Thus, PlxnA1 KO mice may be a suitable model to explore the mechanisms of stereotypic behaviors and PPI deficits fundamental to several neurodevelopmental and psychiatric disorders.

Compliance with ethical standards

All animal care and experimental procedures were conducted 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).

Funding

This work was supported by grants #22590195 and #16K08478 to KY from the Japanese Ministry of Education, Culture, Sports, Science, and Technology (KAKENHI) (http://www.jsps.go.jp/j-grantsinaid/i

ndex.html).

Conflicts of interest

The authors declare no conflicts of interest regarding this work.

CRediT authorship contribution statement

Mst Sharifa Jahan: Investigation, Writing - original draft. **Takuji Ito:** Investigation, Methodology. **Sachika Ichihashi:** Investigation. **Takanobu Masuda:** Investigation. **Md. Eliusur Rahman Bhuiyan:** Investigation, Methodology. **Ikuko Takahashi:** Investigation, Methodology. **Hyota Takamatsu:** Resources. **Atsushi Kumanogoh:** Resources. **Takamasa Tsuzuki:** Investigation, Methodology, Supervision. **Takayuki Negishi:** Investigation, Methodology, Supervision. **Kazunori Yukawa:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Project administration, Software, Supervision, Validation, Visualization, Writing - review & editing.

Acknowledgments

We would like to acknowledge the RIKEN BRC and Dr. Yuchio Yanagawa, Gunma University Graduate School of Medicine, Japan, for generously providing GAD67-GFP knock-in mice and permitting us to use the mice for our study. We would like to thank Drs. Takayoshi Mamiya, Shuhei Yamada, and Masayuki Hiramatsu for providing helpful suggestions to authors.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.ibror.2020.10.004.

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