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Altered synaptic phospholipid signaling in PRG-1 deficient mice induces exploratory behavior and motor hyperactivity resembling psychiatric disorders

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ABSTRACT

Plasticity related gene 1 (PRG-1) is a neuron specific membrane protein located at the postsynaptic density of glutamatergic synapses. PRG-1 modulates signaling pathways of phosphorylated lipid substrates such as lysophosphatidic acid (LPA). Deletion of PRG-1 increases presynaptic glutamate release probability leading to neuronal over-excitation. However, due to its cortical expression, PRG-1 deficiency leading to increased glutamatergic transmission is supposed to also affect motor pathways. We therefore analyzed the effects of PRG-1 function on exploratory and motor behavior using homozygous PRG-1 knockout (PRG-1$^{-/-}$) mice and PRG-1/LPA_2–receptor double knockout (PRG-1$^{-/-}$/$LPA_2^{-/-}$) mice in two open field settings of different size and assessing motor behavior in the Rota Rod test. PRG-1$^{-/-}$ mice displayed significantly longer path lengths and higher running speed in both open field conditions. In addition, PRG-1$^{-/-}$ mice spent significantly longer time in the larger open field and displayed rearing and self-grooming behavior. Furthermore PRG-1$^{-/-}$ mice displayed stereotypical behavior resembling phenotypes of psychiatric disorders in the smaller sized open field arena. Altogether, this behavior is similar to the stereotypical behavior observed in animal models for psychiatric disease of autistic spectrum disorders which reflects a disrupted balance between glutamatergic and GABAergic synapses. These differences indicate an altered excitation/inhibition balance in neuronal circuits in PRG-1$^{-/-}$ mice as recently shown in the somatosensory cortex [38]. In contrast, PRG-1$^{-/-}$/$LPA_2^{-/-}$ did not show significant changes in behavior in the open field suggesting that these specific alterations were abolished when the LPA_2-receptor was lacking. Our findings indicate that PRG-1 deficiency led to over-excitability caused by an altered LPA/LPA_2-R signaling inducing a behavioral phenotype typically observed in animal models for psychiatric disorders.

1. Introduction

Plasticity-related gene 1 (PRG-1) was first described and classified in 2003 as a member of the lipid phosphate phosphatase (LPP) family [1]. It is specifically expressed in neurons of vertebrates and found during postnatal developmental [1]. PRG-1 plays a specific role at excitatory synapses on glutamatergic neurons where it interacts with bioactive phospholipids which interacts with presynaptic LPA_2–receptors [2]. These findings indicate a mechanism for modulation of glutamatergic transmission, which is controlled by PRG-1 and mediated by LPA as a supplemental trigger in addition to the classical molecular machinery at glutamatergic synapses. However, the behavioral consequences of this molecular mechanism are not fully understood [3,4]. PRG-1 is located postsynaptically in glutamatergic but not in GABAergic synapses. PRG-1$^{-/-}$ mice display increased synaptic phospholipid signaling, enhancing presynaptic glutamate release via LPA_2-receptor activation leading to neuronal over-excitability [2]. However, the expression of glutamatergic receptors subunits of NMDA- and AMPA-receptors displayed no difference between PRG-1$^{-/-}$ mice and their wild type (WT) littermates. Also, morphological alterations or changes in inhibitory events were not found in PRG-1$^{-/-}$ mice [2].

Glutamate is an important excitatory neurotransmitter [5] and glutamatergic neurons are widely distributed in brain. Deficits in glutamatergic transmission can affect different functional areas including

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motor behavior and glutamatergic motor pathways including the cortico-striatal pathway, the connection between thalamus and striatum and collateral mossy fibers to cerebellar nuclei [6–9]. Tokumitsu et al. described PRG-1 protein expression in different motor-related brain areas such as the cerebral cortex, the basal ganglia and the cerebellar cortex, [10]. While previous studies described altered processing of sensory inputs in the somatosensory cortex of heterozygous PRG-1 deficient mice [3] there is only scarce information on the effects of PRG-1 deficiency on motor behavior.

In the present study we investigated the role of bioactive phospholipids in the exploration and motor behavior of PRG-1$^{-/-}$ mice and compared them to their WT littermates. PRG-1$^{-/-}$ mice were trained in two open fields (OF) arenas of different sizes which allows to assess different motor activity patterns. In the bigger OF arena PRG-1$^{-/-}$ mice showed increased path length and speed [11,12]. To analyze whether alteration in motor behavior in PRG-1$^{-/-}$ mice was dependent on neuronal over-excitability mediated by presynaptic LPA_2-receptors [2], we also analyzed exploratory and motor behavior in PRG-1$^{-/-}$LPA$_2^{-/-}$ double mutant mice [13–15].

2. Materials and methods

2.1. Animals

Constitutive PRG-1$^{-/-}$ mice were generated as described [2]. To generate a PRG-1 LPA_2 receptor mouse line, heterozygous constitutive PRG-1$^{-/-}$ mice were bred with the LPA_2-receptor mouse line provided by J. Chun [16]. Mice were backcrossed on a C57Bl6 background and female litters were used.

For this study 38 adult PRG-1$^{-/-}$ mice were compared to 38 adult wild type littermates (WT), and 10 adult PRG-1$^{-/-}$LPA$_2^{-/-}$ mice were compared to 8 adult PRG-1$^{+/+}$LPA$_2^{+/+}$ littermates. Mice were singly housed in transparent cages and maintained on a 12/12 h light/dark cycle with food and water ad libitum. All tests were performed during the light phase.

Behavior

To examine aspects of exploratory behavior and activity 20 PRG-1$^{-/-}$ and 20 WT mice were tested in a smaller sized OF arena, the Motility box. Another 18 PRG-1$^{-/-}$ and 18 WT littermates were tested in the larger sized OF. Furthermore, 10 PRG-1$^{-/-}$LPA$_2^{-/-}$ and 8 PRG-1$^{+/+}$LPA$_2^{+/+}$ were examined in the larger OF. Motor behavior was examined using the Rota Rod test with 9 PRG-1$^{-/-}$ comparing with 9 WT littermates and 10 PRG-1$^{-/-}$LPA$_2^{-/-}$ comparing with 8 PRG-1$^{+/+}$LPA$_2^{+/+}$ mice.

2.2. Small OF arena (Motility box)

One apparatus used for the exploration behavior and activity is the Motility box (TSE-Systems, Bad Homburg, Germany). The mice were singly placed in a Plexiglas box (size: 45 × 45 × 45 cm) with 15 infrared sensors installed in x and y dimension at an interval of 3 cm. Additional sensors for movements in z dimension were installed at a height of 10 cm. Illumination was kept at constant level of 125 lx. Mice were tested for 15 min. The box was cleaned after each tested animal. Running time [sec], distance covered [m] and rearing and leaning [frequency] in nine areas of the Motility box were measured automatically by the software Moti-Test-System (TSE-Systems GmbH, Bad Homburg, Germany). These areas include: one central area (center), four corners (corner) and four edge areas between the corners (edge). In the Motility box we examined 20 PRG-1$^{-/-}$ mice and 20 WT littermates.

Results in open field arenas depend on size of the used arena [11,14]. We analyzed behavior in a second, larger arena for PRG-1$^{-/-}$ and PRG-1$^{-/-}$LPA$_2^{-/-}$ mice compared to their wildtype littermates.

2.3. Large OF arena

The second test OF arena was constructed by enclosing a grey plastic floor with same colored planks (size: w x l x h = 85 x 85 x 26 cm). The room was air conditioned (22 °C) and indirect illuminated to provide homogenous diffuse illumination of the arena (250 lx). A video camera (Panasonic CCTV, model: WV-BL200/6) was mounted on the ceiling and was linked to a video recorder (Panasonic SD 430) to record the behavior. Each animal was tested once for 10 min between 8 am and 2 pm. The arena was cleaned after each animal. The recorded videos were analyzed by the software VideoMot 2 (TSE-Systems, Bad Homburg, Germany). Running time [sec], distance covered [m] in nine areas of the OF were measured automatically: one central area (center), four corners (corner) and four edge areas between the corners (edge). Number of rearing and leaning and time of self-grooming were manually detected. In the large OF arena we examined 18 PRG-1$^{-/-}$ mice comparing with 18 WT littermates and 10 PRG-1$^{-/-}$LPA$_2^{-/-}$ mice and 8 PRG-1$^{+/+}$LPA$_2^{+/+}$ littermates.

2.4. Rota rod test

In the Rota Rod test, mice have to maintain balance and keep pace with a rotating rod. The Rota Rod treadmill apparatus (New Rota Rod for Mice, Ugo Basile, Varese, Italy) was divided into 5 separated testing stations. One day before testing, mice were habituated on the apparatus. The animals had to run for 1 min at a speed of 4 rounds/min (rpm) and of 10 rpm with a break of 5 min between the trials. The test run was accelerated from 4 to 40 rpm within 5 min. The total running time and the rpm were manually registered when the mice fell down. Alternatively the run was stopped after 300 s at 40 rpm. The test was performed 3 times with a break of 30 min between the runs. In this test we used 9 PRG-1$^{-/-}$ comparing with 9 WT littermates and 10 PRG-1$^{-/-}$LPA$_2^{-/-}$ comparing with 8 PRG-1$^{+/+}$LPA$_2^{+/+}$ mice.

2.5. Statistical analysis

Statistical analyses were performed using the software SPSS 22 (IBM Corporation, Armonk, New York, USA). After assessing for normality (Kolmogorow-Smirnow-Test) multiple dependent variables in the motility box and OF were analyzed by MANOVA with factor genotype. Samples were compared using the student's *t*-test or Welch-test, after Levene-test of homogeneity of variances and the Kruskal-Wallis Test for nonparametric data (number of leaning and rearing). Value of $p < 0.05$ was regarded as statistically significant.

3. Results

3.1. Small OF (Motility box, 45 cm x 45 cm)

In the Motility box, a small OF setting, PRG-1$^{-/-}$ and WT mice showed significant differences in path length (Fig. 1A). A MANOVA for areas edge, corner and center with genotype as factor revealed differences by tendency between PRG-1$^{-/-}$ and WT mice ($F(2,38) = 2.541$; $p < 0.1$). Post hoc analysis demonstrated significantly longer path lengths for PRG-1$^{-/-}$ at the edges ($t(38) = 2.250$ $p < 0.05$) of the box as well as total path lengths ($t(38) = 2.239$ $p < 0.05$) while path lengths in the corner ($t(38) = 0.649$ $p = 0.52$) and in the center ($t(38) = 1.507$ $p = 0.14$) were not different. However, when assessing the time spent in these regions of the Motility box, no significant differences were observed between genotypes (edge: $t(38) = 1.263$ $p = 0.21$; corner: $t(38) = 1.438$ $p = 0.16$; center: $t(38) = 1.198$ $p = 0.24$; Fig. 1B). These results point to a higher motility in PRG-1$^{-/-}$ mice. However, when assessed for speed, we found a higher speed in PRG-1$^{-/-}$ mice when compared to WT litters (MANOVA: $F(2,38) = 2.941$; $p < 0.1$), which was significantly higher in the edges ($t(38) = 2.175$ $p < 0.05$) and in the corners ($t(38) = 2.375$ $p < 0.05$)