Effect of magnesium sulfate on retinal dopaminergic neurons in rats with 6-OHDA-induced Parkinson's disease

Leyi Huang
Fujian Maternity and Child Health Hospital, Affiliated Hospital of Fujian Medical University
Renxi Lin
Fujian Medical University
Chunying Zhang
Fujian Medical University Union Hosipital
Ling Lin (llinzheng2022@gmail.com)
Fujian Medical University

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Abstract

Parkinson's disease (PD) is one of the most common neurodegenerative diseases. The main pathological features of PD are the degeneration and death of dopamine neurons in the substantia nigra-striatal pathway and the accumulation of Lewy bodies in neurons. In addition to the well-known hallmark symptoms such as resting tremor and muscle rigidity, Parkinson's patients also experience non-motor symptoms such as visual disturbances, hyposmia, and sleep disturbances. At the same time, visual disturbances appear earlier than motor symptoms. The visual dysfunction in early-stage of Parkinson's disease indicates the occurrence and development of the disease. The visual dysfunction in Parkinson's disease is due to the damage of retinal dopamine neurons. This study found that magnesium sulfate supplementation can effectively delay the death of retinal dopamine neurons in 6-OHDA induced rat model of PD and improve the motor symptoms of rats with anxiety-like behavior. Therefore, magnesium sulfate may provide a new option for the early diagnosis and treatment of Parkinson's disease.

1 Introduction

Parkinson's disease (PD) is currently one of the most common neurodegenerative diseases[]. A reduction in dopaminergic neuron number in the substantia nigra-striatal pathway and aggregation of Lewy bodies within neurons are the main pathological features of PD[], and the clinical manifestations of PD include motor symptoms and nonmotor symptoms. Previous studies have shown that visual dysfunction is one of the important early nonmotor symptoms of PD and that it occurs earlier than the motor symptoms of PD, which has important guiding significance for the early diagnosis and treatment of PD[,]. Several studies have found that patients with PD have worse vision, contrast sensitivity, and color discrimination than normal individuals, which is related to the accumulation of α-synuclein in the retina and a reduction in the number of dopamine neurons[]. Although visual dysfunction has important clinical implications for PD, there is currently no available targeted treatment.

In recent years, the beneficial effects of magnesium ion therapy on PD has gradually attracted the attention of the public. Magnesium ions, as the second largest cations in the body, are involved in many important physical activities[]. Magnesium homeostasis in cells is mainly dependent on the regulation of ion channels and magnesium transporters on the cell membrane[]. Several studies have now confirmed that magnesium levels in brain tissues, the cerebrospinal fluid, blood, urine and even hair are significantly lower in PD patients than in normal individuals[,]. Animal experiments have also been revealed that a high-magnesium diet or intraperitoneal injection of magnesium-containing drugs can effectively improve motor symptoms and reduce damage to dopaminergic neurons in PD rats[,]; thus, magnesium homeostasis may have a close relationship with the development of PD. As natural calcium blockers, magnesium ions are able to reduce calcium influx, inhibit reactive oxygen species production, reduce superoxide dismutase activity, reduce oxidative stress and ultimately protect dopaminergic neurons by inhibiting the activity of glutamate N-methyl-D-aspartic acid (NMDA) receptors[]. Commonly used therapeutic drugs for PD, such as levodopa, resagiline, and pramipexole, mainly alleviate symptoms via

dopamine replacement or dopamine receptor agonism[], and magnesium ion treatment is thought to stop disease progression at the source by inhibiting oxidative stress and protecting dopaminergic neurons. Furthermore, it has been found that magnesium ions can effectively maintain the structural stability of the retina and slow the loss of optic nerve cells in glaucoma patients by inhibiting glutamate excitotoxicity[,]. Therefore, magnesium ions may have clinical value in alleviating visual dysfunction in PD patients, and exploring the relationship between magnesium ions, magnesium ion transporters and visual dysfunction in PD is important for the early diagnosis and treatment of PD.

The main objective of this study was to explore the protective effects of magnesium sulfate on retinal dopamine neurons in PD rats, the differential expression of magnesium ion transporters in the retinal tissues of PD rats and the possible mechanisms underlying these changes.

2 Materials And Methods

2.1 Animals

Male specific pathogen-free (SPF) Sprague–Dawley (SD) rats (body weight 280–300 g) were purchased from the Experimental Animal Center of Fujian Medical University. The rats were bred in an SPF environment (12 h light/dark cycle, temperature of 23 ± 2℃, humidity of 55 ± 5%) and fed a normal diet.

2.2 Experimental grouping

The rats were randomized to the 14-day group and 28-day group, and the rats in each group were randomly divided into the control group, control and magnesium supplementation (control/$MgSO_4$) group, PD group, and PD and magnesium supplementation (PD/$MgSO_4$) group.

2.3 6-Hydroxydopamine (6-OHDA) lesioning

Rats were anesthetized with 2% sodium pentobarbital (40 mg/kg) and placed in a stereotaxic instrument (Narishige). After the skin on the head was disinfected with 75% alcohol, an incision was made to expose bregma. 6-OHDA (8 μg/2 μL) was injected into the right MFB according to a rat brain stereotaxic atlas[] (AP: -4.4 mm, ML: -1.4 mm, DV: +8.5 mm) using a modified microinjection device. 6-OHDA was slowly injected via a glass needle (2 μL/8 min), and the glass needle was left in place 5 min before being withdrawn (5 mm/min). The corresponding control groups were injected with 2 μL normal saline (containing 0.2% vitamin C). In addition, rats in the control/$MgSO_4$ group and PD/$MgSO_4$ group received intraperitoneal injection of magnesium sulfate (50 mg/mL) 30 min after surgery and at the same time daily for 14 or 28 days.

2.4 Apomorphine (APO)-induced rotation test

14 days or 28 days after 6-OHDA lesioning, the rats were injected intraperitoneally with APO (0.5 mg/kg) to induce rotational behavior. Rats were considered successful PD rat models if they rotated stably toward the contralateral side for 210 rotations within 30 min (or 7 r/1 min).

2.5 Open-field test

14 days or 28 days after 6-OHDA lesioning, the rats were placed in the center of an open field arena (40 cm*40 cm*35 cm) and allowed to explore freely for 5 min. The total distance traveled, distance traveled in the central zone and activity in the central zone were measured to assess the anxiety of the rats.

2.6 Immunofluorescence staining

The rats were decapitated, and their eyeballs were removed and fixed in ocular fixative solution for 24 h. The tissues were dehydrated, cleared in xylene, embedded in paraffin and cut into 5 μm sections centered on the macula along the sagittal plane. After dewaxing, dehydration and antigen retrieval, paraffin sections were blocked with 5% normal goat serum for 30 min and incubated overnight at 4°C with primary antibodies [anti-tyrosine hydroxylase (TH) antibody (1:300) and anti-glutamate antibody (1:200)]. The next day, after rinsing in PBS, the sections were incubated with CY3-labeled anti-mouse secondary antibodies (1:300) for 60 min at room temperature and quenched with autofluorescence quencher for 30 min. After the nuclei were stained with DAPI, the sections were sealed with anti-fluorescence quenching agent. Five visual fields (×400) were randomly selected for observation with a Leica SP5 laser microscope, and the images were processed with LAS AF Lite software.

2.7 Estimation of magnesium ion content in rat retinal tissues

After anesthesia, the rats were decapitated, their eyes were enucleated, and the preocular segment was removed rapidly on ice. After the addition of a 9× sample volume of PBS, the tissues were ground with a tissue grinder (Servicebio, Wuhan, China). The homogenates were centrifuged for 10 min at 4000 rpm, and the supernatants were collected. Two hundred microliters of working solution was added to the wells of a 96-well plate, and the plate was incubated for 5 min at 37°C. Then, 10 μL of double-distilled water, magnesium standard solution (1.0 mmol/L) and tissue samples were added to the blank wells, standard wells and sample wells, respectively. The mixtures were incubated for 2 min at 37°C. The absorbance of each sample was measured with a microplate reader at a wavelength of 550 nm, and the magnesium ion content was calculated according to the following formula:

$$Mg^{2+}(mmol/L) = \frac{OD(sample) - OD(blank)}{OD(standard) - OD(blank)}$$

2.8 Western blot analysis of magnesium ion transporter expression in retinal tissue

After anesthesia, the rats were decapitated, their eyes were enucleated, and to the preocular segment was removed rapidly on ice. The tissues were homogenized in RIPA buffer and centrifuged at 13000 rpm for 10 min at 4°C. The protein content of the supernatant was estimated using a BCA assay (Beyotime Biotechnology). SDS-sample buffer was added to the remaining supernatant, and the mixture was boiled in a 100°C water bath for 5 min. Ten percent polyacrylamide gel electrophoresis was performed to assess

the expression of magnesium ion transporters. Twenty microliters (25 μg) of each protein sample and 5 μL of protein marker were added to the wells. After the proteins were separated, they were transferred to a PVDF membrane, which was then blocked in 5% skimmed milk for 2 h at room temperature. After washing with PBS, the membrane was incubated with primary antibodies (CNNM2, 1:100, Invitrogen; SLC41A1, 1:100, Novus; MagT1, 1:100, Invitrogen; GAPDH, 1:5000, Abclonal) overnight at 4°C. After washing, the membrane was incubated with secondary antibody (HRP-conjugated goat anti-rabbit, 1:2000; HRP-conjugated goat anti-mouse, 1:5000; Immunoway) for 2 h at room temperature. Finally, the membrane was imaged with a chemiluminescent imager (Gene), and ImageJ software was used for quantitative analysis of the blots.

2.9 Statistical analysis

All statistical analyses were performed with IBM SPSS Statistics 20, and graphs were created with GraphPad Prism 8. All results are expressed as the mean ± standard error of the mean (SEM). One-way analysis of variance (ANOVA) was used for comparisons between multiple groups, and the Wilcoxon signed-rank test was used when the variances within the groups were not homogeneous. Correlations were analyzed using Pearson correlation analysis. A P value of < 0.05 indicates statistical significance.

3 Result

3.1 APO-induced rotation test

The APO-induced rotation test was performed 14 and 28 days after 6-OHDA injection to evaluate the rats' motor symptoms. As shown in Fig. 1, compared with that exhibited by the corresponding control groups, the number of rotations exhibited by the PD groups on 14 days and 28 days was significantly increased ($p < 0.01$). Compared with that exhibited by the PD/$MgSO_4$ group at 14 days, the number of rotations exhibited by the PD/$MgSO_4$ group at 28 days was decreased ($p < 0.01$). 28 days after surgery, the number of rotations exhibited by the PD/$MgSO_4$ group was significantly decreased compared with that exhibited by the PD group ($p < 0.01$).

3.2 Open field test

The open field test was performed to further explore the motor ability and anxiety of the rats. (Fig. 2) The results showed that compared with the control group, the PD group traveled a significantly shorter total distance and a significantly shorter distance in the central zone ($p < 0.05$) 28 days after surgery. Intraperitoneal injection of magnesium sulphate increased the distanced traveled in the central zone by the rats ($p < 0.05$).

3.3 Immunofluorescence

3.3.1 Tyrosine Hydroxylase (TH)

TH is the rate-limiting enzyme for dopamine synthesis, and its fluorescence intensity can be used to assess the survival of retinal dopaminergic neurons.

As shown in Fig. 3–4, at 14 days after surgery, retinal TH fluorescence intensity was decreased by 82.67% ± 0.26% ($p < 0.05$) on the left side (contralateral side) in the PD group compared with the corresponding side in the control group and increased by 298.80% ± 74.28% ($p < 0.01$) and 139.82% ± 42.45% ($p < 0.05$) in the PD/$MgSO_4$ group compared with the corresponding sides in the PD group. At 28 days after surgery, the TH fluorescence intensity in the left retina was decreased by 92.63%±2.23% in the PD group compared with the control group ($p < 0.05$), while the TH fluorescence intensity in the left retina was increased by 166.58%±43.71% in the PD/$MgSO_4$ group compared with the PD group ($p < 0.05$).

3.3.2 Glutamate

Glutamate-induced neuronal excitotoxicity is thought to be a cause of PD. As shown in Fig. 5–6, glutamate content in left retinal tissue was increased by 90.22%±36.96% and 118.00%±68.66% in the PD group compared to the corresponding side in control group at 14 and 28 days postlesioning ($p < 0.05$), while glutamate content in the left retina was decreased by 49.14% ± 7.43% in the PD/$MgSO_4$ group compared to the PD group at 14 days after surgery ($p < 0.05$).

3.5 Magnesium ion content in the retina

As shown in Fig. 7, at 14 days after surgery, the magnesium ion content in the left retina (contralateral side) was decreased by 27.50% ±5.00% in the PD group compared with the corresponding control group ($p < 0.01$), and the magnesium ion content in the remaining groups was not significantly different; however, the magnesium ion content in the left retina showed an increasing trend in the PD/$MgSO_4$ group compared with the PD group. At 28 days after surgery, the magnesium ion content in the right retina (disrupted side) was increased by 84.00% ± 32.00% in the PD/$MgSO_4$ group compared to the PD group ($p < 0.05$).

3.6 Correlation between TH fluorescence intensity and glutamate fluorescence intensity in rat retinal tissue

The correlation between TH fluorescence intensity and glutamate fluorescence intensity in rat retinal tissue was analyzed using Pearson correlation analysis (Fig. 8). The results showed that 14 days after surgery, the TH fluorescence intensity did not correlate with the glutamate fluorescence intensity in the rat retina ($r = 0.6303$, $p > 0.05$, $N = 8$); however, at 28 days after surgery, the TH fluorescence intensity correlated negatively with the glutamate fluorescence intensity in the rat retina ($r = 0.8126$, $p < 0.05$, $N = 8$).

3.7 Western blotting

As shown in Fig. 9, at 14 days after surgery, the protein expression of the magnesium ion transporters MagT1, SLC41A1 and CNNM2 in the left retina (contralateral side) was downregulated by 63.93% ±10.48% ($p < 0.01$), 41.03%±8.97% & 44.29%±9.29% ($p < 0.05$), respectively, in the PD group compared to the corresponding control group. The protein expression of MagT1 and SLC41A1 in the left retina was

upregulated 144.20%±52.63% and 77.27%±29.55%, respectively, in the PD/$MgSO_4$ group compared to the PD group ($p < 0.05$). In addition, SLC41A1 protein expression in the right retina was decreased by 48.00% ±10.00% ($p < 0.01$) in the control/$MgSO_4$ group compared to the control group.

As shown in Fig. 10, at 28 days after surgery, the protein expression of SLC41A1 in the bilateral retina was downregulated by 67.42%±6.82% and 61.00%±8.00% in the PD group and by 60.44%±13.19% and 53.06% ±10.20% in the PD/$MgSO_4$ group compared with the corresponding control group ($p < 0.01$); however, the protein expression levels of MagT1 and CNNM2 were not significantly different among the groups.

Protein expression of the magnesium ion transporters CNNM2, SLC41A1, and MagT1 in rat retina at 14 days after surgery. The relative expression in the right retina of the control group was set as 100%, and relative expression for each group was calculated as (the experimental group gray value/the internal reference gray value)/(the control group gray value/the internal reference gray value).

Protein expression of the magnesium ion transporters CNNM2, SLC41A1, and MagT1 in rat retina at 28 days after surgery. The relative expression in the right retina of the control group was set as 100%, and relative expression for each group was calculated as (the experimental group gray value/the internal reference gray value)/(the control group gray value/the internal reference gray value).

4 Discussion

The retina is a dopamine-rich tissue in the human body. In this study, we used a PD rat model induced by unilateral administration of 6-OHDA to observe the effects of magnesium sulfate on motor symptoms, retinal dopaminergic neuron survival, retinal magnesium ion content and intraretinal magnesium ion transporter expression in PD rats to investigate the changes in retinal magnesium ions and their transporters in PD rats during the progression of PD and to explore the protective effects of magnesium sulfate on retinal dopaminergic neurons in PD rats and the possible underlying mechanisms.

The APO-induced rotation test is currently recognized as a reliable behavioral experiment for the identification of successful PD modeling by unilateral 6-OHDA administration in rats[]. Previous studies by our laboratory found that the number of rotations made by rats in the PD/$MgSO_4$ group was significantly decreased compared with that made by rats in the PD group after 28 days of continuous administration of magnesium sulfate and decreased comparedt with that made by rats in the PD/$MgSO_4$ group after 14 days of magnesium sulfate administration. This indicates that continuous administration of magnesium sulfate in the early stage of neuronal injury can effectively improve motor symptoms in PD rats. This result is generally consistent with the results obtained by Lin et al. in SH-SY5Y human neuroblastoma as well as in animals[,] and validates the proposal made by Shindo's team that magnesium ions exert a neuroprotective effect in an animal model[]. In addition, this study found that PD rats exhibited less activity, had a higher degree of anxiety than normal rats, and had a lack of desire to explore their surroundings in the open field test. Antipova et al[] also found that 6-OHDA-lesioned PD rats show motor retardation and slowness, suggesting that PD rats may show psychological symptoms such

as anxiety in addition to impairment of motor function. Moreover, this study found that early and continuous administration of magnesium sulfate can improve motor symptoms and reduce the anxiety of PD rats to some extent. Magnesium was also found to have a mitigating effect on motor and psychological symptoms in PD rats in a study by Hajizade et al[].

In the retina, dopamine is synthesized by subtype A18 cells without long protrusions or cells of the inner plexiform layer[], which are mainly found in the inner plexiform and inner nuclear layers of the retina[] and can affect the activity of a variety of cells within the retina[23]. TH is the rate-limiting enzyme for dopamine synthesis, and its level is indicative of dopamine levels[]. This study found that the TH fluorescence intensity in the inner plexiform and inner nuclear layers of the left retina (contralateral to the lesion) was reduced by more than 80% and 90%, respectively, in the PD group compared with the control group at 14 and 28 days after lesioning and that the TH fluorescence intensity in bilateral retina increased significantly after magnesium sulfate supplementation, suggesting that the loss of retinal dopaminergic neurons in PD rats increased with the progression of PD. This result is similar to that the findings of Lin et al[18], who showed that a single injection of 6-OHDA into the MFB destroyed dopaminergic neurons not only in the substantia nigra pars compacta (SNc) but also in the contralateral retina, while magnesium sulfate had a protective effect on retinal dopaminergic neurons[].

Glutamate is an important central neurotransmitter, and the accumulation of α-synuclein in the brains of PD patients leads to excessive glutamate accumulation and excitotoxicity and triggers overload of Ca^{2+} and excessive loss of magnesium ions in mitochondria through continuous activation of NMDA glutamate receptors, which eventually leads to the death of dopaminergic neurons[11]; thus, glutamate excitotoxicity is one of the main pathogenic mechanisms of PD[]. In this study, at 14 and 28 days after 6-OHDA lesioning, there was a significant decrease in the number of dopaminergic neurons in the left retina in the PD group compared with the control group, glutamate content was also significantly increased in the PD group compared to the control group, and TH content in the PD rat retina was inversely proportional to glutamate content. Zhang et al.[] also found that glutamate levels are significantly higher in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced PD model mice than in control mice and that reducing glutamate excitotoxicity can reverse dopaminergic neuron degeneration. Chotibut et al.[] observed that the number of TH-positive neurons in the striata of PD rats decreases as glutamate clearance decreases, while the number of TH-positive neurons in rats rebounds significantly after intravenous administration of ceftriaxone, which increases glutamate clearance. Salvatore's team[] similarly found that increased glutamate content in the nigrostriatal pathway of PD rats is accompanied by excessive Ca^{2+} efflux, activation of calpain, cleavage of downstream proteins, and eventual death of dopaminergic neurons and that inhibition of glutamate release can effectively protect dopaminergic neurons. The present study on PD rat retinal tissues, which are dopamine-rich, similar to the nigrostriatal pathway, reached a similar conclusion as the abovementioned experiment. It is clear that the relationship between glutamate and dopaminergic neurons in the retina, a dopamine-rich region, may be similar to that in the nigrostriatal pathway.

Moreover, in this study, it was found that continuous administration of magnesium sulfate to PD rats for 14 or 28 days decreased glutamate content in the retina and significantly improved the survival of dopaminergic neurons. This result supports Lambuk et al.'s[15] conclusion that magnesium can alleviate the loss of dopaminergic neurons due to glutamate excitotoxicity in the PD mouse retina, which may be due to the exogenous magnesium's ability to elevate the magnesium ion concentration in the cerebrospinal fluid, to improve motor symptoms and slow dopaminergic neuron degeneration. The protective effect of magnesium on dopaminergic neurons may be related to inhibition of glutamate excitotoxicity, as evidenced by the efficacy of exogenous magnesium described above[]. However, notably, some researchers have found that the changes in magnesium ion levels in the brain tissues and blood of animal models of neurodegenerative diseases are not obvious and that the neuroprotective effect of magnesium ions is limited; these differences may be related to the route of magnesium administration, dose and analysis methods[,].

Most scholars believe that magnesium can reduce α-synuclein aggregation in the brains of PD patients and decrease the neurotoxicity of glutamate by inhibiting NMDA glutamate receptors, thus protecting dopaminergic neurons[,]; thus, the local magnesium ion concentration in tissues may be related to neurotoxicity and dopaminergic neuron survival. According to the results of this study, the magnesium ion concentration in the left retinas of PD rats decreased to a certain degree at 14 days after injection of 6-OHDA but returned to normal at 28 days. Strugeon et al.[] examined the magnesium ion content in the brains of PD patients and normal subjects by atomic spectroscopy and found that the magnesium ion content in the brain tissues of PD patients was significantly lower than that in the brain tissues of normal subjects; these results are consistent with the findings of the present study, in which the magnesium ion content in the rat retina was examined, suggesting that magnesium ion content is altered in response to oxidative stress and neurodegeneration in tissues to some extent. However, in some studies[13], the magnesium ion content in brain tissues and body fluids was not significantly different between PD patients and normal subjects; while these differences may be related to the analysis methods used, they also indicate that the magnesium ion content in tissues may fluctuate less over a certain period of time under compensatory and homeostatic regulation of the body.

In the present study, after 6-OHDA injection into the right MFB, the magnesium ion content in the retinas of the rats in the PD group showed a more pronounced decrease on the left side than on the right side; this result is consistent with the findings of Meng[], who concluded that 6-OHDA has a more marked effect on dopamine and melatonin content in the contralateral eye in an animal model, possibly because 6-OHDA disrupts the dopamine neuron membrane in the retina on the contralateral side through the optic chiasm, leading to intracellular Ca^{2+} overload and magnesium ion loss, which eventually triggers dopaminergic neuron death[], and is in agreement with the phenomenon that approximately 98% of the retinal ganglion cells project to the contralateral midbrain superior colliculus[].

Magnesium ion transporters are the main factors that regulate intracellular magnesium homeostasis[7]. In the present study, at 14 days after lesioning, MagT1, SLC41A1 and CNNM2 protein expression in the

left retina was downregulated to some degree in the PD group compared to the control group, while MagT1 and SLC41A1 protein expression was upregulated in the magnesium sulfate-treated group compared to the PD group on the corresponding side, and CNNM2 protein expression tended to be increased. Shindo et al.[] found that CNMM2 mRNA expression was significantly downregulated in MPP^+-induced PD cell model (PC12 cells). Additionally, Lin et al[18] found that 6-OHDA downregulates the expression of several intracellular magnesium transporters (MagT1, SLC41A1, CNNM2) in a 6-OHDA-induced PD cell model and that supplementation with magnesium sulfate increases the expression of these magnesium transporters in a 6-OHDA-induced rat model at 14 days after lesioning. The same trend was found for SLC41A1 protein expression in the striata of rats in the PD group[19]. The above results are consistent with the results of the present study, in which the levels of magnesium ion transporters in the retinal tissues of PD rats were examined, suggesting that MagT1, SLC41A1 and CNNM2 are more sensitive to 6-OHDA toxicity in the acute phase of injury and that the expression levels of these transporters in the retina are basically consistent with those in the striatum, the main target of PD, and can respond to the progression of PD to a certain extent; in addition, the appropriate dose of magnesium sulfate can upregulate the expression of MagT1 and CNNM2 and activate SLC41A1 to reverse-mediate the inward flow of magnesium, playing a role in increasing the intratissue magnesium ion content and alleviating the symptoms of PD rats[].

Notably, at 28 days after 6-OHDA injection, SLC41A1 protein expression was significantly downregulated in the bilateral retina in the PD and PD/$MgSO_4$ groups compared with the respective control groups, while the protein expression levels of MagT1 and CNNM2 were no longer significantly different between the groups. This result suggests that magnesium homeostasis in rats may be maintained by multiple magnesium transporters and that magnesium transporters may have different functions, with MagT1 and CNNM2 mediating the inward flow of magnesium ions and SLC41A1 mediating the outward flow of magnesium ions[12]. As PD progresses, there may be some balance between the different magnesium transporters; downregulation of SLC41A1 expression may slow intracellular magnesium ion loss, whereas MagT1 and TRPM7 are functionally similar [12] and may complement each other. Thus, the relative stability of retinal MagT1 and CNNM2 protein expression in the PD group may be due to changes in the expression of the other magnesium transporters.

In conclusion, the present study found that magnesium sulfate has a protective effect on dopamine neurons in the retinas of PD rats, which has potential clinical value for the treatment of visual dysfunction in PD patients. Furthermore, various Mg transporters may have different functions in PD and jointly maintain the homeostasis of intracellular magnesium ion concentrations. In pathological states, the expression of different magnesium transporters may vary, and their mechanisms of action still need to be further investigated.

Conclusion

In this study, we found that unilateral 6-OHDA-induced rat model of PD mainly destroyed contralateral retinal dopamine neurons, which partially down-regulated magnesium ion transporter expression and caused magnesium ion deficiency in the retina. Magnesium sulfate supplementation alleviated motor symptoms and anxiety-like behavior in PD rats, upregulated partial magnesium ion transporter expression, alleviated magnesium ion deficiency in the retina, reduced glutamate release, and protected retinal dopamine neurons in PD rats.

Declarations

Anthor Contributions *Ling Lin contributed to the study conception and design. Material preparation, data collection and analysis were performed by Leyi Huang, Chunying Zhang and Renxi Lin. The first draft of the manuscript was written by Leyi Huang and Renxi Lin. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.*

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Data Avilability All data generated or analyzed during this study are available from the corresponding author on reasonable request.

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References

1. Pringsheim T, Jette N, Frolkis A, et al (2014) The Prevalence of Parkinson's Disease: A Systematic Review and Meta-analysis. Movement Disorders. https://doi.org/10.1002/mds.25945
2. Sveinbjornsdottir S (2016) The clinical symptoms of Parkinson’s disease. Journal of Neurochemistry. https://doi.org/10.1111/jnc.13691
3. Xiangdan L, Yuling L, Haina S (2019) Evaluation of olfactory function and related factors in patients with Parkinson's disease. Chinese Journal of Neuroimmunology and Neurology. (Chinese)
4. Lijun L, Xiaohong L (2019) Research progress of visual dysfunction in Parkinson's disease. Chinese Journal of Geriatric heart brain and vessel disease. (Chinese)

5. Huang LY, Lin L (2020) The basic and clinical studies of retinal changes in Parkinson's disease. International Review of Ophthalmology. (Chinese)
6. Yamanaka R, Shindo Y, Oka K (2019) Magnesium Is a Key Player in Neuronal Maturation and Neuropathology. International Journal of Molecular ences. https://doi.org/10.3390/ijms20143439
7. Tomita A, Zhang M, Fei J, et al (2017) ATP-dependent modulation of MgtE in Mg2+ homeostasis. Nature Communications.. https://doi.org/10.1038/s41467-017-00082-w
8. Bocca B, Alimonti A, Senofonte O, et al (2006) Metal changes in CSF and peripheral compartments of parkinsonian patients. Journal of the Neurological Sciences. https://doi.org/10.1016/j.jns.2006.05.007
9. Yasui M, Kihira T, Ota K (1992) Calcium, magnesium and aluminum concentrations in Parkinson's disease. NeuroToxicology.
10. Shen YL, Dai L, Tian HB, et al (2019) Treatment Of Magnesium-L-Threonate Elevates The Magnesium Level In The Cerebrospinal Fluid And Attenuates Motor Deficits And Dopamine Neuron Loss In A Mouse Model Of Parkinson's disease.[J] .Neuropsychiatr Dis Treat. https://doi.org/10.2147/NDT.S230688
11. Shindo Y, Yamanaka R, Hotta K, et al (2020) Inhibition of Mg2+ Extrusion Attenuates Glutamate Excitotoxicity in Cultured Rat Hippocampal Neurons. Nutrients. https://doi.org/10.3390/nu12092768
12. De Baaij JHF, Hoenderop JGJ, Bindels RJM (2015) Magnesium in Man: Implications for Health and Disease. Physiological Reviews. https://doi.org/10.1152/physrev.00012.2014
13. Miyake Y, Fukushima W, Tanaka K, et al (2011) Dietary intake of metals and risk of
14. inson's disease: A case-control study in Japan. Journal of the Neurological
15. s. https://doi.org/10.1016/j.jns.2011.03.035
16. Li Z, Tian T (2017) Light Therapy Promoting Dopamine Release by Stimulating Retina in Parkinson Disease. JAMA Neurol. https://doi.org/10.1001/jamaneurol.2017.1906
17. Lambuk L, Jafri AJ, Arfuzir NN, et al (2017) Neuroprotective Effect of Magnesium Acetyltaurate Against NMDA-Induced Excitotoxicity in Rat Retina. Neurotox Res. https://doi.org/10.1007/s12640-016-9658-9
18. inos G, Watson C (2007) The rat brain in stereotaxic coordinates, 3rd edn. San Diego: academic press. Psychopharmacology.
19. Nishijima H,Kimura T, Mori F, et al (2021) Effects of Aging on Levo-Dihydroxyphenylalanine-Induced Dyskinesia in a Rat Model of Parkinson's Disease. Front Aging Neurosci. https://doi.org/10.3389/fnagi.2021.650350
20. Lin L, Ke ZL, lv MQ, et al (2017) Effects of MgSO4 and magnesium transporters on 6-hydroxydopamine-induced SH-SY5Y cells. Life Sci. https://doi.org/10.1016/j.lfs.2016.12.010
21. Lin L, Yan MZ, Wu B, et al (2018) Expression of magnesium transporter SLC41A1 in the striatum of 6-hydroxydopamine-induced parkinsonian rats. Brain Res Bull. https://doi.org/10.1016/j.brainresbull.2018.08.019

22. Shindo Y, Yamanaka R, Suzuki K, et al (2015) Intracellular magnesium level determines cell viability in the MPP(+) model of Parkinson's disease. Biochim Biophys Acta. https://doi.org/10.1016/j.bbamcr.2015.08.013
23. Antipova V, Holzmann C, Hawlitschka A, et al (2021) Antidepressant-Like Properties of Intrastriatal Botulinum Neurotoxin-A Injection in a Unilateral 6-OHDA Rat Model of Parkinson's Disease. Toxins. https://doi.org/10.3390/toxins13070505
24. Hajizade GS, Sheikh M, Dehghan SM, et al (2017) The Association Between Effective Dose of Magnesium and Mild Compulsive Exercise on Spatial Learning, Memory, and Motor Activity of Adult Male Rats. Biol Trace Elem Res. https://doi.org/10.1007/s12011-016-0916-8
25. Lin L, Tong Y (2011) The role of retinal dopamine system in visual information transmission. International Review of Ophthalmology. (Chinese)
26. Jackson CR, Capozzi M, Dai H, et al (2014) Circadian Perinatal Photoperiod Has Enduring Effects on Retinal Dopamine and Visual Function. J Neurosci. https://doi.org/10.1523/JNEUROSCI.4887-13.2014
27. Feng Y, Ma JJ, Yuan LP (2020) β-Methylphenylalanine exerts neuroprotective effects in a Parkinson's disease model by protecting against tyrosine hydroxylase depletion. J Cell Mol Med. https://doi.org/10.1111/jcmm.15571
28. Sarre S,Yuan H, Jonkers N, et al (2004) In vivo characterization of somatodendritic dopamine release in the substantia nigra of 6-hydroxydopamine-lesioned rats. J Neurochem. https://doi.org/10.1111/j.1471-4159.2004.02471.x
29. Iovino L, Tremblay ME, Civiero L (2020) Glutamate-induced excitotoxicity in Parkinson's disease: The role of glial cells. J Pharmacol Sci. https://doi.org/10.1016/j.jphs.2020.07.011
30. Zhang Y, He X, Meng X, et al (2017) Regulation of glutamate transporter trafficking by Nedd4-2 in a Parkinson’s disease model. Cell Death Dis. https://doi.org/ doi.org/10.1038/cddis.2016.454
31. Chotibut T, Davis RW, Arnold JC, et al (2014) Ceftriaxone increases glutamate uptake and reduces striatal tyrosine hydroxylase loss in 6-OHDA Parkinson's model. Mol Neurobiol. https://doi.org/10.1007/s12035-013-8598-0
32. Salvatore MF, Davis RW, Arnold JC, et al (2012) Transient striatal GLT-1 blockade increases EAAC1 expression, glutamate reuptake, and decreases tyrosine hydroxylase phosphorylation at ser(19). Experimental Neurology. https://doi.org/10.1016/j.expneurol.2012.01.012
33. Shen YL, Dai L,Tian HB, et al (2019) Treatment Of Magnesium-L-Threonate Elevates The Magnesium Level In The Cerebrospinal Fluid And Attenuates Motor Deficits And Dopamine Neuron Loss In A Mouse Model Of Parkinson's disease. Neuropsychiatric disease and treatment. https://doi.org/10.2147/NDT.S230688
34. Koç ER, Ilhan A, Zübeyde A, et al (2015)A comparison of hair and serum trace elements in patients with Alzheimer disease and healthy participants. Turk J Med Sci. https://doi.org/10.3906/sag-1407-67

35. Rajput K, Afridi HI, Kazi TG, et al (2021) Sodium, Potassium, Calcium, and Magnesium in the Scalp Hair and Blood Samples Related to the Clinical Stages of the Parkinson's Disease. Biol Trace Elem Res. https://doi.org/ 10.1007/s12011-020-02399-y
36. Golts N, Snyder H, Frasier M, et al (2002) Magnesium inhibits spontaneous and iron-induced aggregation of alpha-synuclein. J Biol Chem, 2002. https://doi.org/ 10.1074/jbc.M107866200
37. Sarre S, Yuan H, Jonkers N, et al (2004) In vivo characterization of somatodendritic dopamine release in the substantia nigra of 6-hydroxydopamine-lesioned rats. J Neurochem. https://doi.org/ 10.1111/j.1471-4159.2004.02471.x
38. Sturgeon M, Wu P, Cornell R (2016) SLC41A1 and TRPM7 in magnesium homeostasis and genetic risk for Parkinson's disease. J Neurol Neuromedicine. https://doi.org/ 10.29245/2572.942X/2016/9.1102
39. Tao M. (2012) Relationships between the retina-midbrain-striatum-pineal gland axis and Parkinson's disease. (Doctoral dissertation, Fujian Medical University). (Chinese)
40. Qing Z, Shenggang S, Yunjian Z, et al (2005) Determination and significance of calcium and magnesium ions in erythrocytes of monkey model of Parkinson's disease. Journal of Brain and Nervous Disease. (Chinese)
41. Forrester J, Peters A (1967) Nerve fibres in optic nerve of rat. Nature. https://doi.org/ 10.1038/214245a0
42. Shindo Y, Yamanaka R, Suzuki K, et al (2016) Altered expression of Mg2+ transport proteins during Parkinson's disease-like dopaminergic cell degeneration in PC12 cells. Biochim Biophys Acta. https://doi.org/ 10.1016/j.bbamcr.2016.05.003
43. Kolisek M, Sponder G, Mastrototaro L, et al (2013) Substitution p.A350V in Na+/Mg2+ Exchanger SLC41A1, Potentially Associated with Parkinson's Disease, Is a Gain-of-Function Mutation. PLoS ONE. https://doi.org/ 10.1371/journal.pone.0071096

Figures

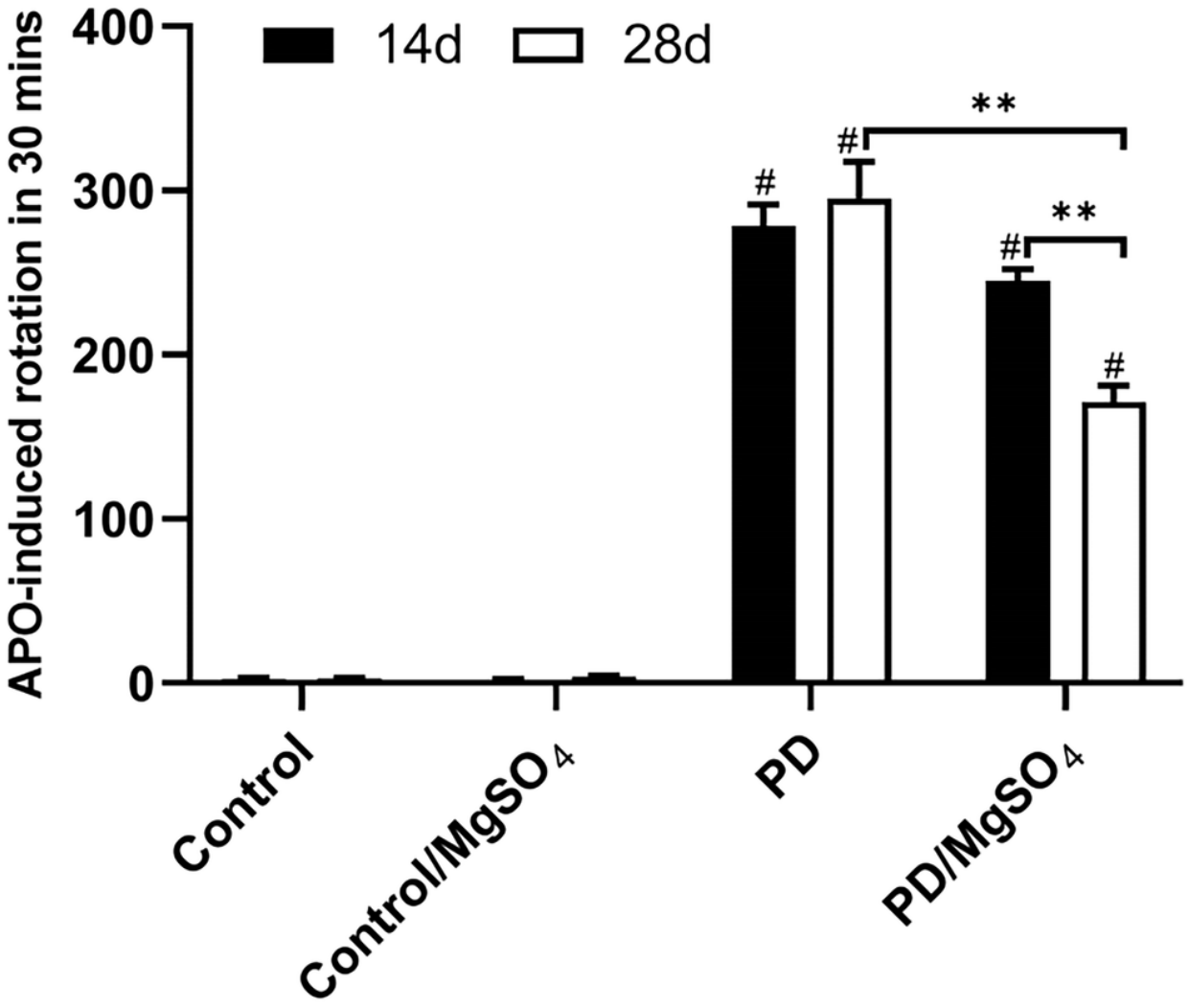

Figure 1

The APO-induced rotation test was performed 14 days and 28 days after surgery. **#** indicates the PD group vs. the control group, $p<0.01$; ** indicates the PD/$MgSO_4$ group vs. the PD group at 28 days after surgery and the PD/$MgSO_4$ group at 28 days vs. the PD/$MgSO_4$ group at 14 days, $p<0.01$; N=6.

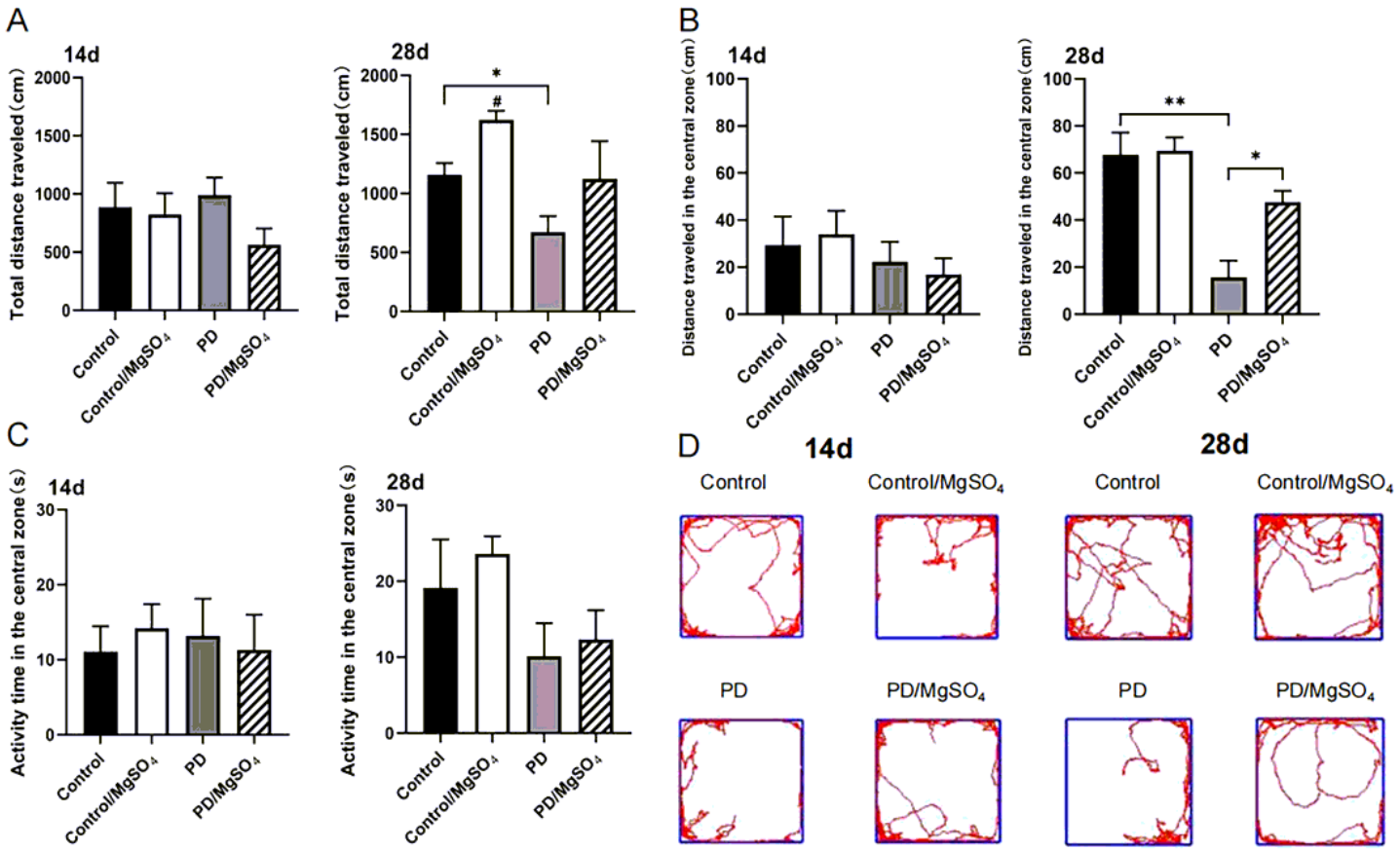

Figure 2

(A) Total distance traveled. **(B)** Distance traveled in the central zone. **(C)** Activity time in the central zone. **(D)** Activity traces.

 indicates the PD group vs. the control group at 28 days after surgery, $p<0.01$;

 indicates the PD group vs. the control group and the PD/$MgSO_4$ group vs. the PD group at 28 days after surgery, $p<0.05$; N=5.

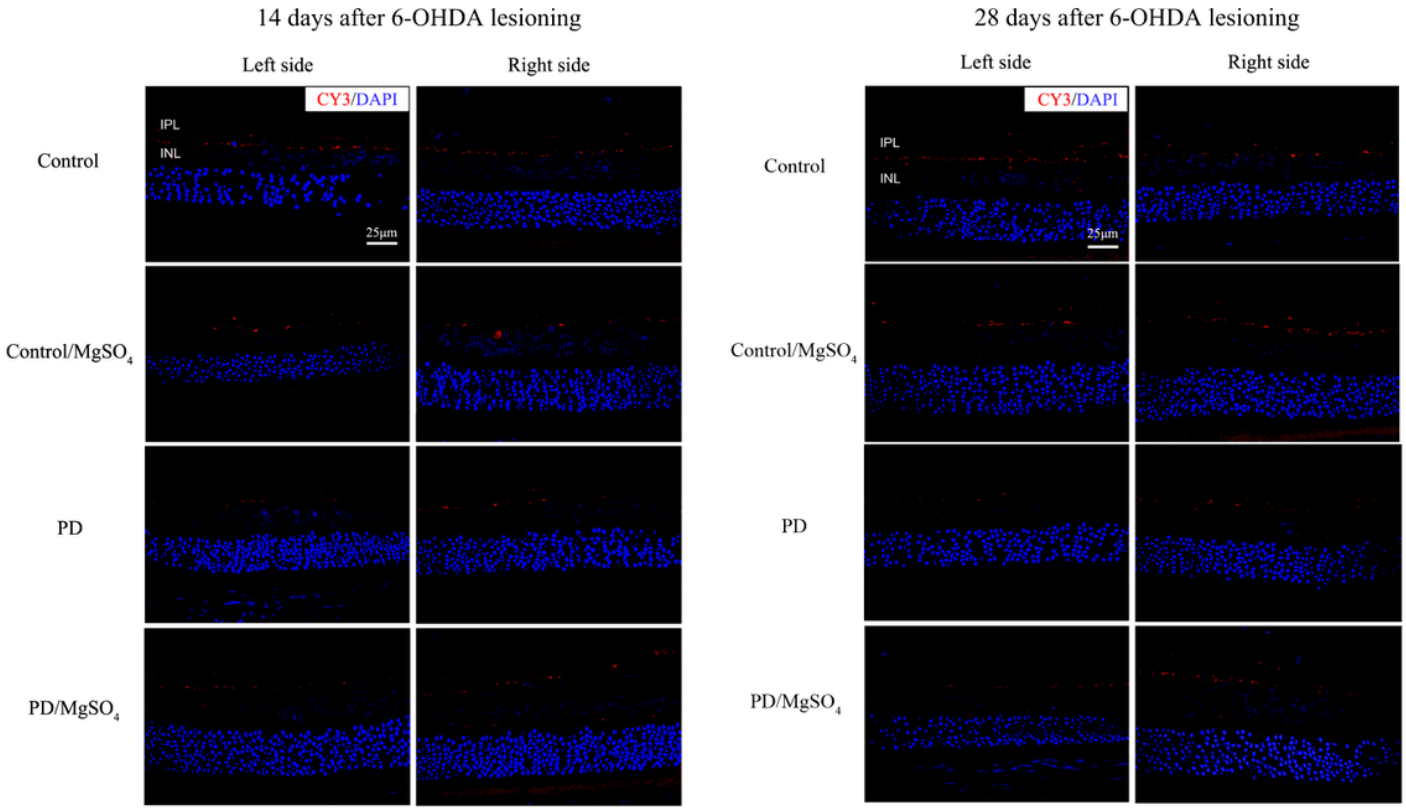

Figure 3

TH fluorescence was observed under a laser confocal microscope (bar=25 μm) (red: CY3, blue: DAPI). IPL: inner plexiform layer; INL: inner nuclear layer.

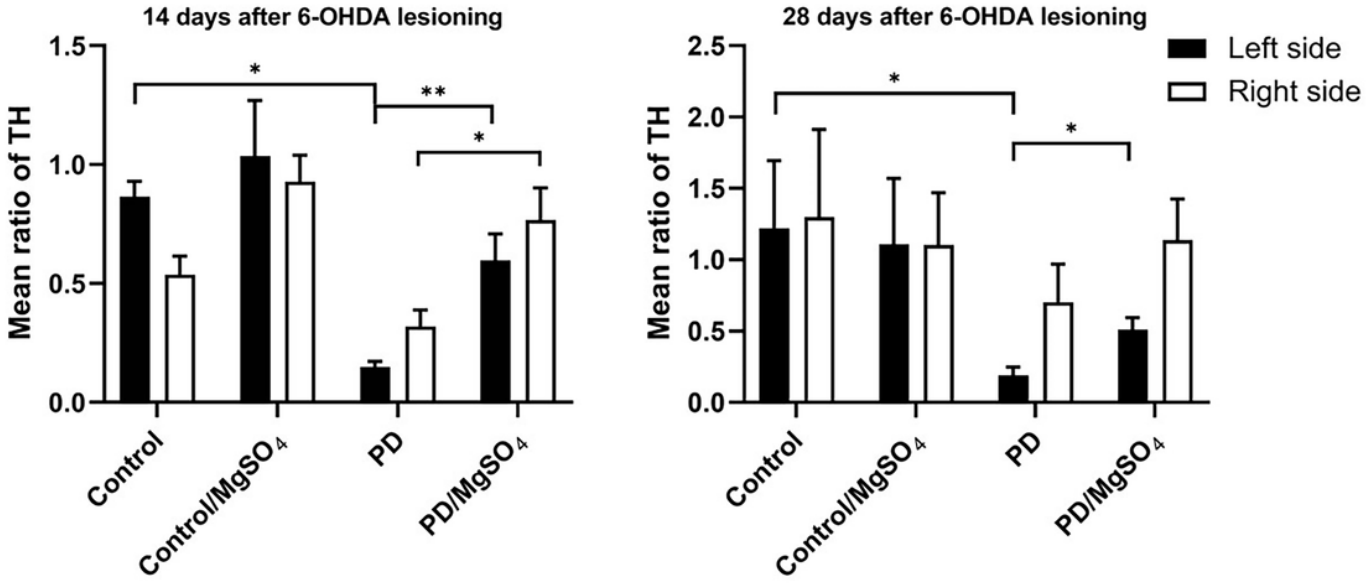

Figure 4

TH immunofluorescence staining intensity in rat retinal tissue. ▯ indicates the PD group vs. the control group (left side) and the PD/$MgSO_4$ group vs. the PD group (right side) at 14 days after surgery and the PD group vs. the control group (left side) and the PD/$MgSO_4$ group vs. the PD group (left side) at 28 days after surgery, $p<0.05$. ▯▯ indicates the PD/$MgSO_4$ group vs. the PD group (left side) at 14 days after surgery, $p<0.01$; N=5.

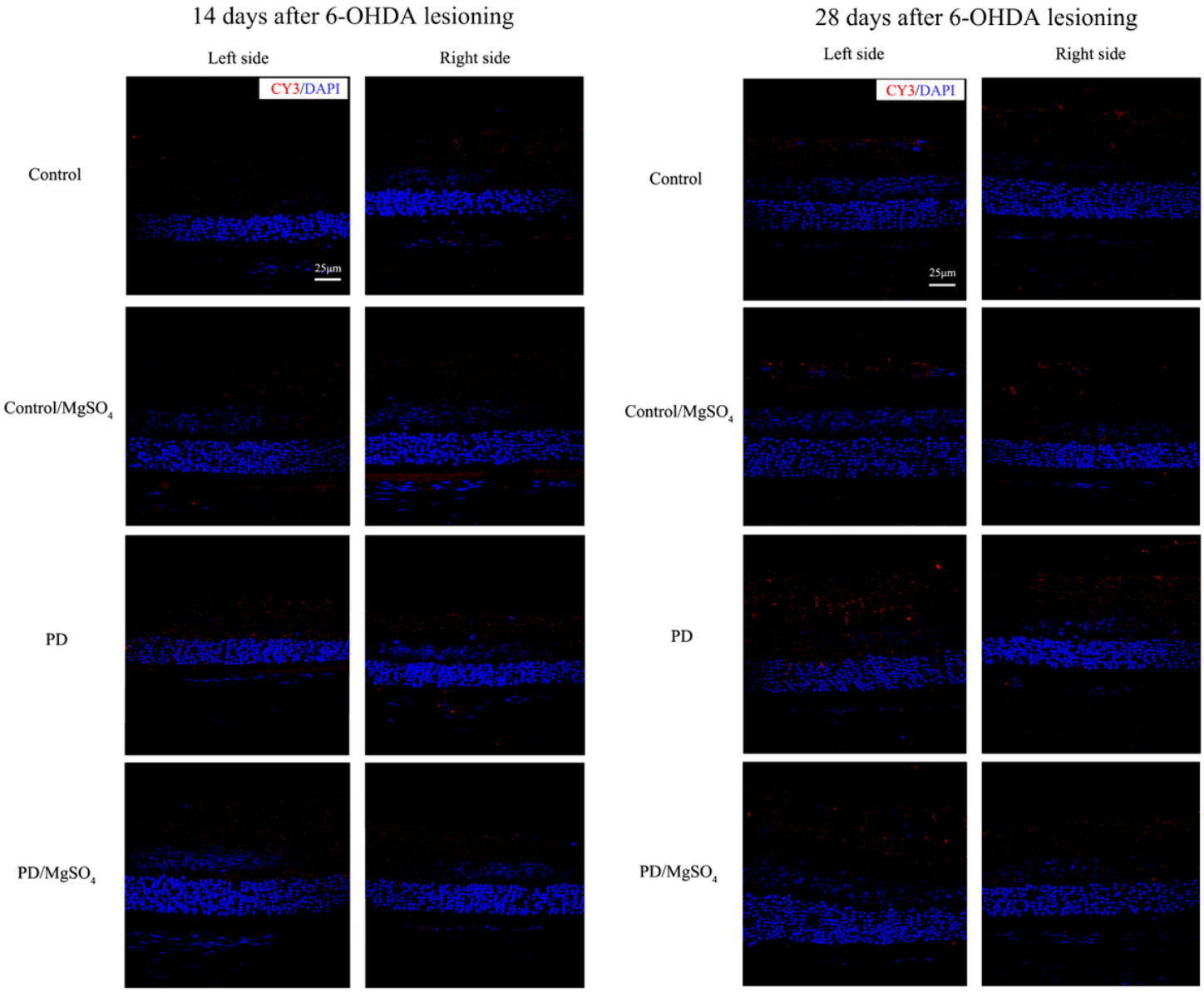

Figure 5

Glutamate fluorescence was observed under a laser confocal microscope (bar=25 μm) (red: CY3, blue: DAPI). IPL: inner plexiform layer; INL: inner nuclear layer.

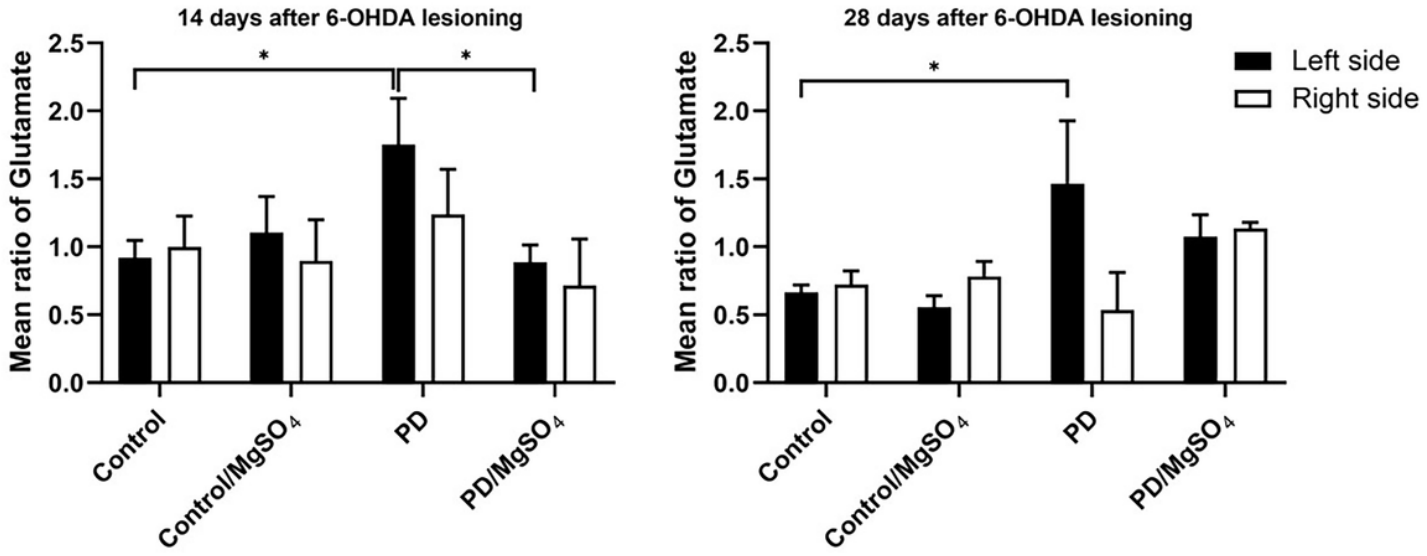

Figure 6

Glutamate immunofluorescence staining intensity in rat retinal tissue.

 indicates the PD group vs. the control group (left side) and the PD/$MgSO_4$ group vs. the PD group (left side) at 14 days after surgery and the PD group vs. the control group (left side) at 28 days after surgery, $p<0.05$; N=4.

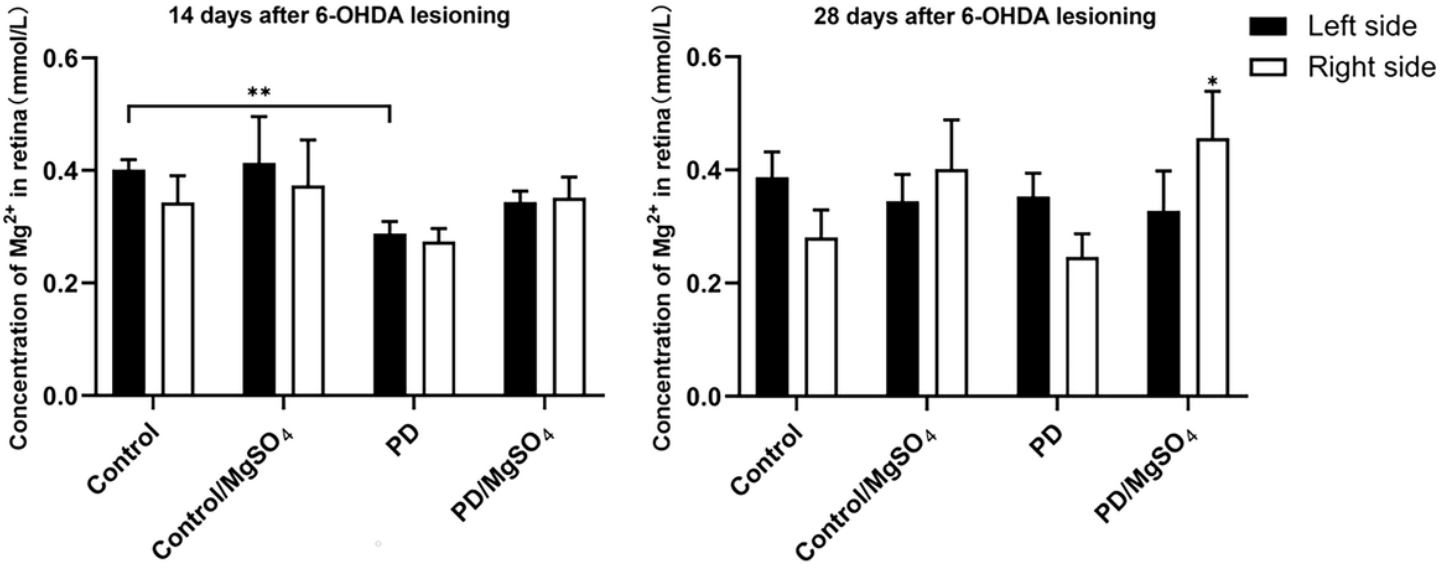

Figure 7

Magnesium ion content in rat retinal tissue at 14 and 28 days after surgery determined with a magnesium ion kit. indicates the left side in the PD group vs. the left side in the control group at 14 days

after surgery, p<0.01; □ indicates the right side in the PD/$MgSO_4$ group vs. the right side in the PD group at 28 days after surgery, *p*<0.05; N=6.

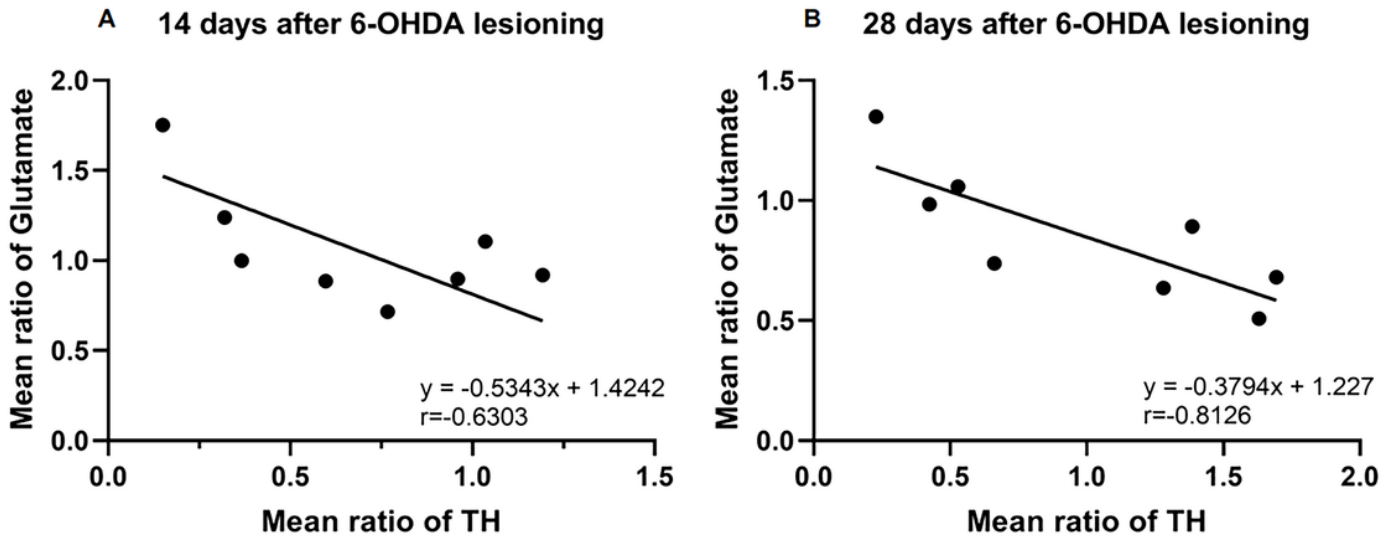

Figure 8

Correlation analysis of the TH fluorescence intensity with the glutamate fluorescence intensity in the rat retina at 14 days (**A**, *p*>0.05, N=8) and 28 days (**B**, *p*<0.05, N=8) after surgery.

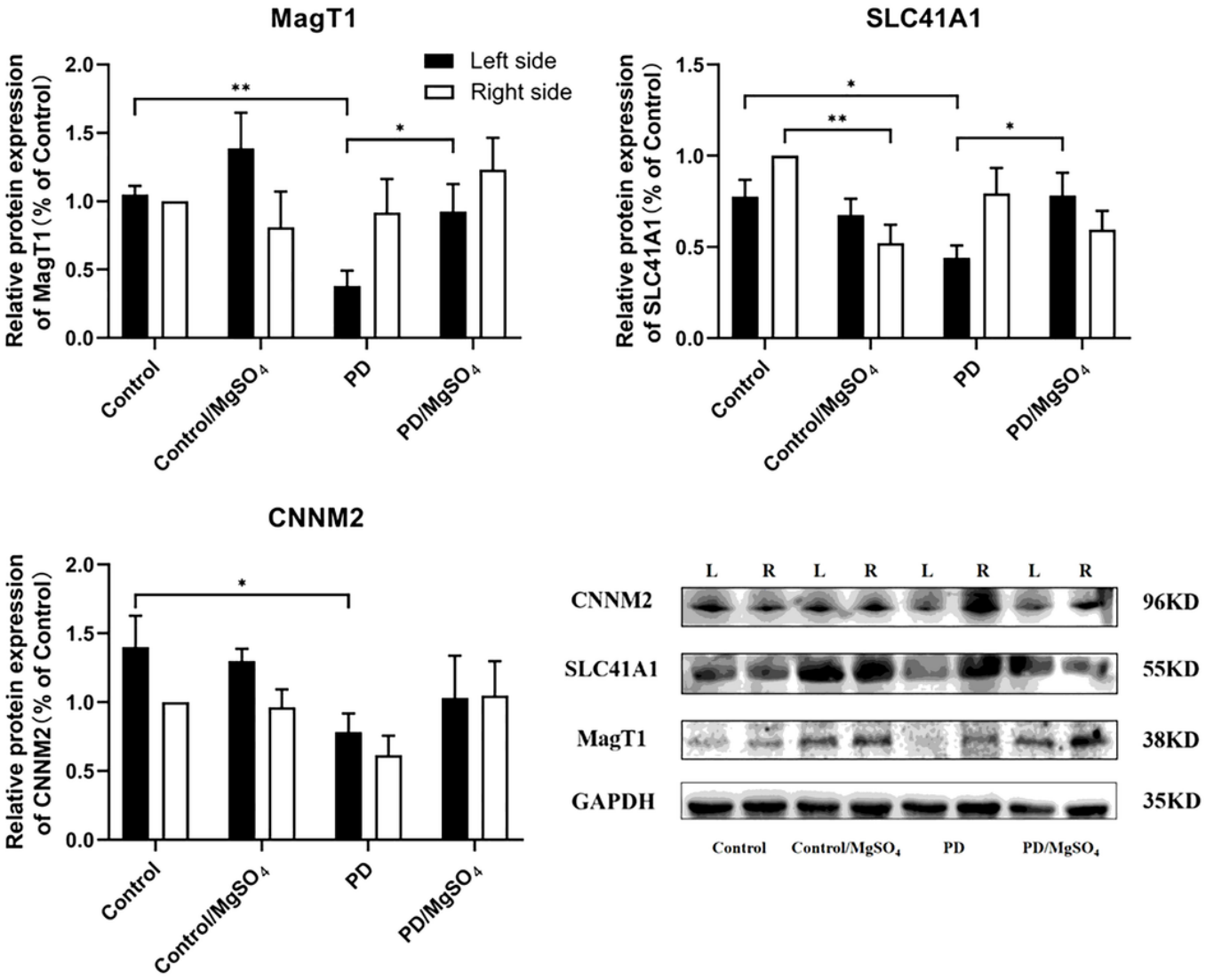

Figure 9

Protein expression of the magnesium ion transporters CNNM2, SLC41A1, and MagT1 in rat retina at 14 days after surgery. The relative expression in the right retina of the control group was set as 100%, and relative expression for each group was calculated as (the experimental group gray value/the internal reference gray value)/(the control group gray value/the internal reference gray value).

(A) Western blot analysis of the expression of the magnesium ion transporters CNNM2, SLC41A1, and MagT1 in the rat retina in each group. **(B)** * indicates $p<0.05$; ** indicates $p<0.01$; N=6.

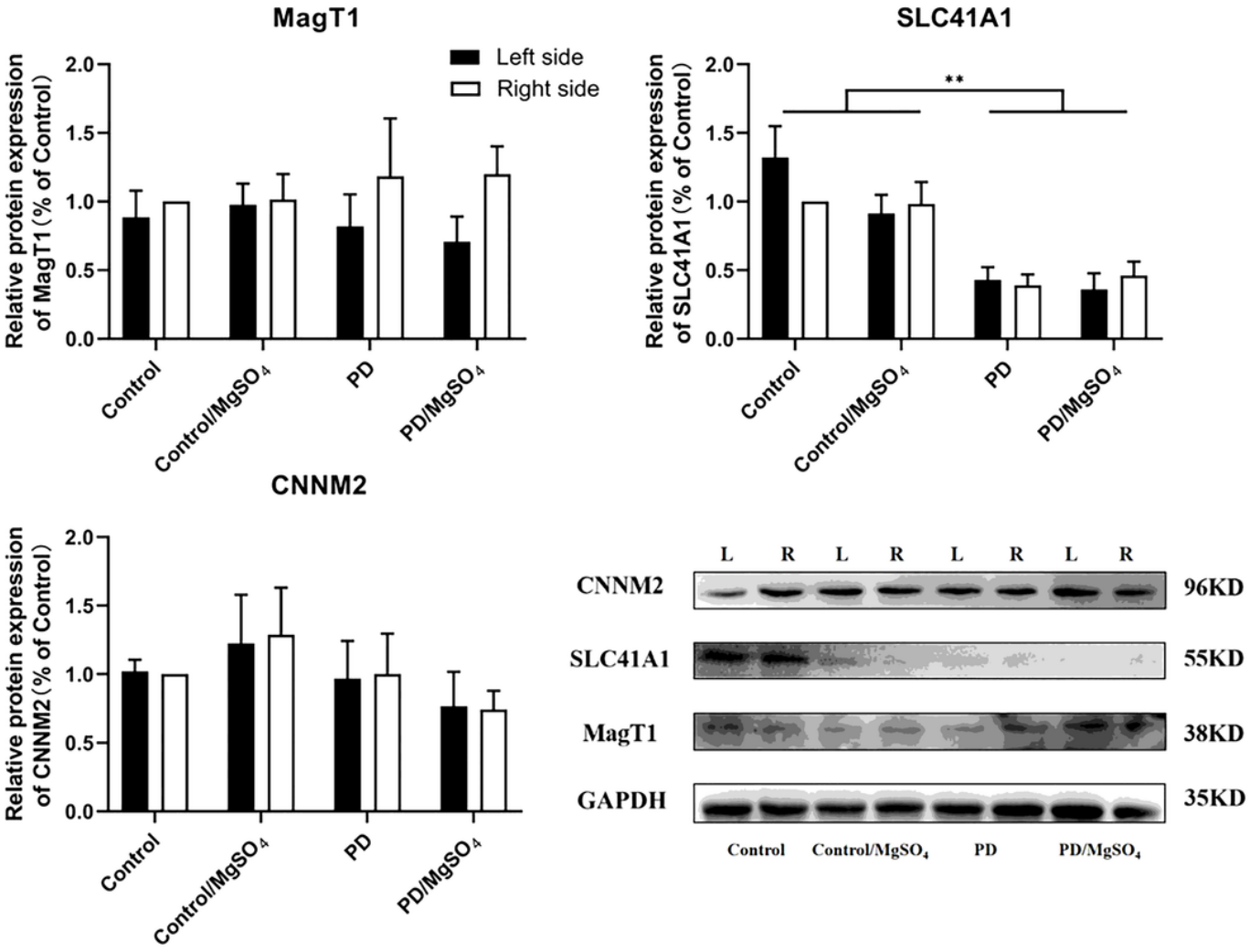

Figure 10

Protein expression of the magnesium ion transporters CNNM2, SLC41A1, and MagT1 in rat retina at 28 days after surgery. The relative expression in the right retina of the control group was set as 100%, and relative expression for each group was calculated as (the experimental group gray value/the internal reference gray value)/(the control group gray value/the internal reference gray value).

(A) Western blot analysis of the expression of the magnesium ion transporters CNNM2, SLC41A1, and MagT1 in the rat retina in each group. **(B)** □ indicates $p<0.05$; □□ indicates $p<0.01$; N=6.