

Temporal Interference Stimulation Alleviates Motor Deficits in MPTP-Induced Parkinson's Disease Mice via Modulation of Striatal Synaptic Plasticity

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Abstract

Purpose

Temporal interference (TI) stimulation is a promising approach for modulating deep brain regions, but its therapeutic effects and molecular basis in Parkinson's disease (PD) remain unclear. We investigated striatal TI stimulation in an MPTP-induced mouse model of PD.

Methods

MPTP-treated mice received seven days of striatal TI stimulation. Motor performance, nigrostriatal dopaminergic markers, dopamine and glutamate receptor-associated proteins, and striatal proteomic changes were evaluated using behavioral tests, immunohistochemistry, Western blotting, and proteomic analysis.

Results

TI stimulation improved motor coordination, gait, pole test performance, and wire suspension ability. TI attenuated MPTP-associated reductions in tyrosine hydroxylase immunoreactivity in the substantia nigra pars compacta and striatum, restored striatal DRD2 protein abundance, and increased DRD1 expression. Proteomic analysis revealed significant enrichment of postsynaptic density-related proteins toward the MPTP-TI group. Consistent with these findings, TI attenuated MPTP-associated elevations in GluN2B, GluR1, and GluR2 and partially restored PSD95 expression, whereas GluN2A remained unchanged.

Conclusions

Striatal TI stimulation alleviates parkinsonian motor deficits and is associated with dopaminergic and postsynaptic molecular changes. These findings provide a preclinical basis for further investigation of TI-based interventions for PD.

1. Introduction

Parkinson's disease (PD) is a common progressive neurodegenerative disorder characterized clinically by bradykinesia, resting tremor, rigidity, and postural instability (Ben-Shlomo et al., 2024). These motor manifestations are primarily associated with degeneration of dopaminergic neurons in the substantia nigra pars compacta (SNpc) and the consequent depletion of dopamine (DA) in the striatum (Morris et al., 2024). Although DA replacement therapy provides substantial symptomatic benefit, its efficacy may fluctuate with disease progression and long-term treatment can be accompanied by motor complications (Yadav & Kumar, 2022). Moreover, pharmacological treatment does not fully correct the distributed basal ganglia circuit abnormalities associated with progressive nigrostriatal dysfunction (Hayes, 2019). These limitations highlight the need for neuromodulator approaches capable of targeting deep motor circuits and producing sustained functional improvement.

The striatum is a major input nucleus of the basal ganglia and integrates dopaminergic signals from the SNpc with glutamatergic inputs from the cerebral cortex(Zhao et al., 2022). Under physiological conditions, DA facilitates movement by activating dopamine D1 receptors (DRD1) on direct-pathway spiny projection neurons while suppressing indirect-pathway neurons through dopamine D2 receptors (DRD2)(Ayon-Olivas et al., 2023; Channer et al., 2023). DA depletion in PD weakens direct-pathway facilitation and reduces dopaminergic inhibition of the indirect pathway, thereby contributing to impaired movement initiation and execution. At the same time, altered corticostriatal glutamatergic transmission may further disrupt striatal output(Gerfen & Surmeier, 2011). NMDA and AMPA receptors mediate excitatory synaptic transmission and interact with postsynaptic scaffold proteins, including postsynaptic density protein 95 (PSD95), to organize receptor localization, intracellular signaling, and activity-dependent synaptic adaptation(Peng et al., 2023). Abnormal receptor abundance, subunit composition, or postsynaptic organization may therefore contribute to maladaptive corticostriatal signaling and motor dysfunction in PD(Harris et al., 2024; Vicente et al., 2020).

Temporal interference (TI) stimulation is an emerging neuromodulation strategy in which two high-frequency electric currents with a small frequency difference interact to generate a lower-frequency amplitude-modulated electric field within deep tissue(Dmochowski & Bikson, 2017). By adjusting electrode placement, current intensity, and carrier frequencies, TI has the potential to preferentially engage deep brain targets while limiting stimulation of superficial regions. Previous work from our group demonstrated that repeated striatal TI stimulation enhanced motor performance in healthy mice and was accompanied by neuroplasticity-related changes in neurotransmitter regulation, synaptic proteins, dendritic spines, and synaptic ultrastructure(Liu et al., 2023). The translational potential of TI has also been supported by clinical studies from our group. TI stimulation targeting the subthalamic region or globus pallidus internus produced acute improvements in motor symptoms, particularly bradykinesia and tremor, in patients with PD(Yang et al., 2024, 2025). These findings indicate that TI can modulate motor-related basal ganglia regions in both experimental animals and patients. Despite these encouraging findings, the molecular responses underlying TI-mediated motor improvement in the parkinsonian brain remain insufficiently characterized. In particular, it is unclear whether striatal TI stimulation influences the nigrostriatal dopaminergic system, DA receptor expression, or the postsynaptic organization of glutamatergic signaling in PD.

In the present study, we investigated the effects of striatal TI stimulation in a subacute MPTP-induced mouse model of PD. Motor performance was evaluated using beam balance test, gait test, pole test, and wire suspension test, while regional c-Fos expression was examined to assess engagement of the intended striatal target. Nigrostriatal dopaminergic alterations were evaluated using TH immunohistochemistry, and striatal DRD1 and DRD2 expression was assessed by immunohistochemistry and Western blotting. We further examined NMDA and AMPA receptor subunits and PSD95 and performed independent proteomic analyses of control and MPTP-treated striata, followed by GO and preranked GSEA. We hypothesized that striatal TI stimulation would ameliorate MPTP-induced motor dysfunction and that this improvement would be associated with preservation of dopaminergic signaling and coordinated remodeling of postsynaptic protein networks.

2. Results

2.1 Confirm the PD model has been successfully established

The experimental timeline and TI electrode configuration are shown in Fig. 1. Mice received MPTP or saline for 10 consecutive days, followed by sham or active TI stimulation for 7 days. Before TI treatment, MPTP exposure reduced TH immunoreactivity in both the SNpc and striatum, confirming successful establishment of nigrostriatal dopaminergic injury. Relative TH signal was reduced in the SNpc (MPTP: 0.74 ± 0.02 vs control: 1.00 ± 0.11 , $P < 0.01$) and striatum (MPTP: 0.81 ± 0.04 vs control: 1.00 ± 0.02 , $P < 0.01$).

2.2 The accuracy of TI stimulation

Immunofluorescence analysis of c-Fos expression was performed after a single session of TI stimulation to assess the regional activation pattern (Fig. 2). The number of c-Fos-positive cells was significantly increased in the stimulated striatum of control mice compared with sham-stimulated controls (Con-TI: 1.55 ± 0.17 vs. Con-Sham: 1.00 ± 0.20 , $P < 0.05$). In contrast, only sparse c-Fos-positive cells were detected in the examined cortical regions, and cortical c-Fos expression did not show a comparable increase following TI stimulation. These findings indicate that TI stimulation preferentially engaged the intended striatal target without inducing widespread activation in the sampled cortical areas.

2.3 TI stimulation relieved motor impairment in the MPTP-treated mice

2.3.1 Beam balance test

The time points for behavioral tests are shown in Fig. 1a. Figure 3b and 3d displays the beam balance test trends following 1, 3, and 7 sessions of TI stimulation. Notably, a single TI session was sufficient to reduce both the times of feet slipped (MPTP-TI: 4.28 ± 1.11 s; MPTP-Sham group: 7.78 ± 1.65 s; $P < 0.01$) and the time required to cross the balance beam (MPTP-TI: 9.83 ± 1.97 s; MPTP-Sham group: 14.83 ± 4.09 s; $P < 0.01$). By the 7 days of stimulation, these parameters had recovered to levels comparable to those observed in the control group. The results of the beam balance test on day 7 are depicted in Fig. 3a and 3c. The time mice cross the balance beam is significantly increased in MPTP-Sham group compared with Con-Sham group on day 7 (MPTP-Sham: 11.56 ± 1.83 s; Con-Sham group: 4.89 ± 0.53 s; $P < 0.01$). Mice in MPTP-TI spend less time cross the balance beam than MPTP-Sham on day 7 (MPTP-TI group: 5.22 ± 0.71 s; MPTP-Sham: 11.56 ± 1.83 s, $P < 0.01$). No significant difference in climbing down time was found between the Con-TI and Con-Sham groups on day 7 (Con-TI group: 5.78 ± 0.50 s, Con-Sham group: 4.89 ± 0.53 s, $P = 0.194$) despite an observed decreasing trend in the Con-TI group. The

number of times the feet slipped is significantly increased in MPTP-Sham group compared with Con-Sham group on day 7 (MPTP-Sham: 5.33 ± 2.49 s; Con-Sham group: 0.39 ± 0.30 s; $P < 0.01$). Mice in MPTP-TI spend less feet slipped times than MPTP-Sham on day 7 (MPTP-TI group: 1.06 ± 0.40 s; MPTP-Sham: 5.33 ± 2.49 s, $P < 0.01$).

2.3.2 Gait test

The stride rate and the stride width of hind paws are useful to evaluate motor functions in PD mice. Figure 3f and 3h displays the gait test trends following 1, 3, and 7 sessions of TI stimulation. Notably, a single TI session was sufficient to produce a positive trend in stride rate, although not statistically significant rate (MPTP-TI: 11.01 ± 1.57 s; MPTP-Sham group: 8.93 ± 1.17 s; $P = 0.172$), while simultaneously eliciting a significant improvement in stride width (MPTP-TI: 2.41 ± 0.16 s; MPTP-Sham group: 2.82 ± 0.66 s; $P < 0.01$). The results of the Gait test on day 7 are depicted in Fig. 3e and 3g. The rate mice cross the runway is significantly reduced in MPTP-Sham group compared with Con-Sham group on day 7 (MPTP-Sham: 9.50 ± 1.90 s; Con-Sham group: 15.56 ± 1.74 s, $P < 0.01$). Mice in MPTP-TI spend faster rate cross the runway than MPTP-Sham on day 7 (MPTP-TI group: 16.53 ± 3.59 s; MPTP-Sham: 9.50 ± 1.90 s, $P < 0.01$). No significant difference in climbing down time was found between the Con-TI and Con-Sham groups on day 7 (Con-TI group: 17.50 ± 4.42 s, Con-Sham group: 15.56 ± 1.74 s, $P = 0.336$), despite an observed faster rate in the Con-TI group. The stride width of hind paws is significantly increased in MPTP-Sham group compared with Con-Sham group on day 7 (MPTP-Sham: 2.71 ± 0.07 s; Con-Sham group: 2.21 ± 0.13 s, $P < 0.01$). Mice in MPTP-TI spend shorter stride width than MPTP-Sham on day 7 (MPTP-TI group: 2.36 ± 0.12 s; MPTP-Sham: 2.71 ± 0.07 s, $P < 0.01$).

2.3.3 Pole test

We adopt the pole test to assess the effects of TI stimulation on the motor balance of PD model mice. Figure 3j displays the pole test trends following 1, 3, and 7 sessions of TI stimulation. Notably, a single TI session was sufficient to reduce the time locomotor (MPTP-TI: 9.83 ± 1.58 s; MPTP-Sham group: 14.61 ± 2.84 s; $P < 0.01$). The results of the pole test on day 7 are depicted in Fig. 3i. The time mice spent climbing down the pole is significantly increased in MPTP-Sham group compared with Con-Sham group on day 7 (MPTP-Sham: 9.28 ± 2.38 s; Con-Sham group: 6.00 ± 0.77 s, $P < 0.01$). Mice in MPTP-TI spend less time climbing down the pole than MPTP-Sham on day 7 (MPTP-TI group: 5.11 ± 1.03 s; MPTP-Sham: 9.28 ± 2.38 s, $P < 0.01$). And the Con-TI group is faster than Con-Sham group on day 7 (Con-TI group: 4.00 ± 0.77 s, Con-Sham group: 6.00 ± 0.77 s, $P < 0.05$).

2.3.4 Wire suspension test

Wire suspension Test was used to evaluate the muscular strength of mice. Figure 3l displays the wire suspension test trends following 1, 3, and 7 sessions of TI stimulation. Notably, a single TI session was sufficient to improve the grip strength (MPTP-TI: 4.11 ± 0.57 s; MPTP-Sham group: 2.17 ± 0.63 s; $P < 0.01$). The results of the wire suspension test on day 7 are depicted in Fig. 3k. The grip strength is significantly reduced in MPTP-Sham group compared with Con-Sham group on day 7 (MPTP-Sham: 3.17

± 0.57 s; Con-Sham group: 4.78 ± 0.25 s, $P < 0.01$). Mice in MPTP-TI showed a significant enhancement of grip strength than MPTP-Sham on day 7 (MPTP-TI group: 4.89 ± 0.16 s; MPTP-Sham: 3.17 ± 0.57 s, $P < 0.01$). No significant difference in grip strength was found between the Con-TI and Con-Sham groups on day 7 (Con-TI group: 4.89 ± 0.16 s, Con-Sham group: 4.78 ± 0.25 s, $P = 0.60$).

2.4 TI stimulation attenuates nigrostriatal dopaminergic loss

TH immunohistochemistry was used to assess the nigrostriatal dopaminergic system after the 7-day intervention (Fig. 4). Relative TH signal was lower in MPTP-Sham than Con-Sham mice in the SNpc (0.75 ± 0.07 vs 1.00 ± 0.08 , $P < 0.01$) and striatum (0.81 ± 0.03 vs 1.00 ± 0.04 , $P < 0.01$). TI stimulation increased TH signal in MPTP-treated mice in both regions (SNpc: 0.97 ± 0.07 vs 0.75 ± 0.07 , $P < 0.01$; striatum: 0.94 ± 0.05 vs 0.81 ± 0.03 , $P < 0.01$). In control mice, TI did not significantly change SNpc TH signal but modestly increased striatal TH immunoreactivity. These findings indicate that TI was associated with preservation or recovery of nigrostriatal dopaminergic markers in the MPTP model.

2.5 TI stimulation induces proteomic remodeling in the control striatum

Con-sham and Con-TI striata were analyzed in an independent 4D-DIA proteomic experiment (Fig. 5). Using a candidate threshold of absolute fold change ≥ 1.2 and nominal $P < 0.05$, 184 TI-responsive proteins were identified, including 115 increased and 69 decreased proteins. Nine proteins remained significant after Benjamini-Hochberg correction: KCNA3, EIF4E2, CSNK1G2, CACNG4, BBIP1, CBFB, PTK2B, EGR1, and GTF2B. CACNG4 and PTK2B were increased, whereas KCNA3 was decreased after TI stimulation. CACNG4 is an auxiliary AMPA-receptor regulatory protein (Radin et al., 2018), and PTK2B is a calcium-sensitive kinase linked to neuronal signaling (De Luca et al., 2025a). GO over-representation analysis of the candidate protein set highlighted cytoskeletal regulation, distal axonal components, and neuronal GTPase-related functions. Together, these findings suggest that TI stimulation in the healthy striatum is associated with changes in receptor regulation, intracellular signaling, neuronal excitability, and structural remodeling.

2.6 Proteomic profiling reveals coordinated remodeling of postsynaptic protein networks after TI stimulation in MPTP-treated mice

To characterize the molecular response of the PD striatum to TI stimulation, proteomic profiling was independently performed in the MPTP-Sham and MPTP-TI groups. (Fig. 6). Using an absolute fold-change threshold of ≥ 1.2 and a nominal P value < 0.05 , 156 candidate TI-responsive proteins were identified, including 78 proteins with increased abundance and 78 with decreased abundance in the MPTP-TI group. Among these proteins, HECA and TRAF3IP3 were increased, whereas NCF1 was

decreased after TI stimulation and remained significant following Benjamini–Hochberg correction. Pathway-level alterations were further examined using preranked GSEA based on all 7,620 unique protein-associated gene symbols quantified in the dataset.

Positive normalized enrichment scores (NES) indicated enrichment toward the MPTP-TI end of the ranked list, whereas negative NES values indicated enrichment toward MPTP-Sham. The postsynaptic density gene set was significantly enriched in the MPTP-TI group (NES = 1.539, nominal $P = 0.00129$, BH-adjusted $P = 0.0486$; Fig. 6d). The leading-edge subset included DLG4/PSD95, GRIA1, GRIA2, GRIN1, GRIN2A, GRIN2B, SHANK-family proteins, and CAMK2 isoforms, indicating coordinated alterations in proteins involved in postsynaptic organization and glutamatergic signaling. Translation at the postsynapse also showed positive enrichment toward the MPTP-TI group (NES = 2.006, nominal $P = 0.00150$; Fig. 6e), although this term did not remain significant after BH correction (adjusted $P = 0.112$) and was therefore interpreted as an exploratory trend. Negative enrichment trends were observed for the tricarboxylic acid cycle, glutathione metabolism, and voltage-gated potassium-channel activity; however, none of these pathways passed the 5% BH-adjusted significance threshold. Collectively, these findings indicate that TI stimulation is associated with coordinated remodeling of postsynaptic protein networks in the parkinsonian striatum.

2.7 TI stimulation modulates striatal dopamine receptor expression

DRD1 and DRD2 expression was examined by immunohistochemistry and Western blotting (Fig. 7). Immunohistochemical signals for both receptors were lower in MPTP-Sham than Con-Sham mice (DRD1: 0.76 ± 0.06 vs 1.00 ± 0.02 , $P < 0.01$; DRD2: 0.87 ± 0.02 vs 1.00 ± 0.02 , $P < 0.01$). TI increased both signals in MPTP-treated mice (DRD1: 0.95 ± 0.03 vs 0.76 ± 0.06 , $P < 0.01$; DRD2: 0.99 ± 0.01 vs 0.87 ± 0.02 , $P < 0.01$). Western blotting confirmed a reduction in DRD2 protein abundance in MPTP-Sham mice compared with Con-Sham mice (0.67 ± 0.14 vs. 1.00 ± 0.24 , $P < 0.01$), followed by a significant increase after TI stimulation (0.94 ± 0.18 vs. 0.67 ± 0.14 , $P < 0.05$). DRD1 protein abundance was also significantly higher in MPTP-TI mice than in MPTP-Sham mice (1.12 ± 0.05 vs. 1.01 ± 0.07 , $P < 0.05$). In control mice, TI stimulation similarly increased DRD1 protein abundance (1.16 ± 0.12 vs. 1.00 ± 0.10 , $P < 0.01$). These results indicate that TI stimulation modifies striatal DA receptor expression, with the most consistent biochemical evidence observed for recovery of DRD2 protein abundance in MPTP-treated mice.

2.8 TI stimulation normalizes glutamate receptor-associated abnormalities and partially restores PSD95

MPTP treatment altered glutamate receptor subunits and postsynaptic scaffold proteins in the striatum (Fig. 8). GluN2B was increased in MPTP-Sham compared with Con-Sham mice (1.31 ± 0.23 vs 1.00 ± 0.26 , $P < 0.01$) and decreased after TI stimulation (0.94 ± 0.06 vs 1.31 ± 0.23 , $P < 0.01$). GluN2A did not differ significantly among the four groups. GluR1 was elevated by MPTP (1.95 ± 0.15 vs 1.00 ± 0.28 , $P < 0.01$) and reduced by TI (1.32 ± 0.23 vs 1.95 ± 0.15 , $P < 0.01$). GluR2 was also elevated in MPTP-Sham

mice (1.52 ± 0.22 vs 1.00 ± 0.08 , $P < 0.01$) and reduced after TI (0.87 ± 0.16 vs 1.52 ± 0.22 , $P < 0.01$). PSD95 was reduced in MPTP-Sham mice (0.80 ± 0.03 vs 1.00 ± 0.10 , $P < 0.01$) and partially restored after TI stimulation (0.92 ± 0.07 vs 0.80 ± 0.03 , $P < 0.05$). These targeted biochemical findings are consistent with the GSEA evidence for coordinated postsynaptic density remodeling.

3. Discussion

The present study demonstrates that striatal TI stimulation improves motor performance in MPTP-treated mice and is accompanied by preservation of nigrostriatal dopaminergic markers and modulation of striatal dopamine receptor expression. At the network level, proteomic GSEA revealed significant enrichment of postsynaptic density-related proteins toward the MPTP-TI group. Consistent with this unbiased proteomic finding, targeted biochemical analyses showed that TI attenuated MPTP-associated alterations in GluN2B, GluR1, and GluR2 and partially restored PSD95 expression. Together, these findings identify postsynaptic protein-network remodeling as a plausible molecular correlate of TI-mediated motor improvement. To our knowledge, this study provides the first preclinical evaluation of TI stimulation in a mouse model of PD.

TI stimulation was initially introduced as a strategy for modulating deep brain structures through the interaction of two high-frequency electric fields, which generate a lower-frequency envelope within the targeted region (Grossman et al., 2017). Previous work from our group showed that seven consecutive days of striatal TI stimulation enhanced motor performance in healthy mice and was associated with neuroplasticity related changes, including altered synaptic protein expression, neurotransmitter regulation, dendritic spine remodeling, and improved synaptic ultrastructure (Liu et al., 2023). These preclinical observations have subsequently been extended to patients with PD. In a randomized, double-blind, sham-controlled pilot study, our group found that a single session of TI stimulation targeting the right globus pallidus internus (Gpi) improved overall motor performance in patients with mild PD, with particularly evident effects on bradykinesia and tremor (Yang et al., 2025). A further pilot study targeting the subthalamic region also reported acute improvements in PD motor symptoms (Yang et al., 2024). More recently, an individualized 130-Hz TI protocol targeting the subthalamic region was evaluated in a randomized, double-blind, sham-controlled crossover study involving 30 patients with early-to-mid-stage PD. Active stimulation produced a higher responder rate than sham stimulation and reduced MDS-UPDRS-III scores immediately and for up to 60 min after stimulation, with the most consistent improvements observed in bradykinesia and tremor (Xu et al., 2026). Although these studies targeted different basal ganglia nodes, together they support the therapeutic potential of TI for modulating motor circuits in PD. The present animal study extends these clinical findings by providing molecular evidence of the striatal responses associated with TI stimulation.

The regional c-Fos pattern observed in the present study supports preferential engagement of the intended striatal target. A single TI session significantly increased c-Fos-positive cells in the stimulated striatum, whereas only sparse labeling was observed in the sampled cortical regions. This pattern is consistent with the ability of the selected electrode configuration to preferentially modulate the deep

target without inducing widespread cortical activation. Functionally, TI stimulation improved several complementary dimensions of motor performance, including balance, gait, bradykinesia-related pole performance, and wire suspension ability. Improvements were already evident after the first stimulation session and were maintained or expanded over the 7-day intervention. This rapid behavioral response is broadly consistent with the acute motor benefits observed in previous clinical TI studies, whereas the progressive improvement across repeated sessions suggests that cumulative neural adaptation may also contribute to the therapeutic response.

The behavioral recovery was accompanied by attenuation of MPTP-associated reductions in TH immunoreactivity in both the SNpc and striatum. The SNpc to striatum dopaminergic projection is essential for the regulation of basal ganglia motor output, and preservation of TH signals suggests that TI stimulation maintained or restored the dopaminergic phenotype within this pathway. The absence of a significant change in SNpc TH expression in control mice further suggests that the effect was not simply a nonspecific global increase in TH expression. In contrast, the modest increase in striatal TH immunoreactivity in control mice may indicate that TI can also modify terminal dopaminergic function under physiological conditions. These findings provide a dopaminergic basis for the observed improvements in motor coordination and movement initiation.

The DA receptor results further connect TI stimulation with striatal basal ganglia signaling. Under physiological conditions, DA promotes direct-pathway signaling through DRD1 and suppresses indirect-pathway spiny projection neurons through DRD2 (Vicente et al., 2020). DA depletion in PD consequently reduces direct-pathway facilitation while removing dopaminergic inhibition of the indirect pathway, contributing to impaired movement¹⁸. In the present study, MPTP reduced DRD1 and DRD2 immunoreactivity, and TI stimulation increased both receptor signals. Western blotting provided particularly consistent evidence for DRD2, which was reduced after MPTP exposure and restored toward control levels by TI stimulation. Recovery of DRD2 expression may improve the responsiveness of indirect-pathway neurons to residual dopaminergic input and thereby reduce excessive indirect-pathway influence on motor output. DRD1 abundance was also increased in MPTP-TI mice and in control mice following TI stimulation, suggesting that TI may facilitate dopaminergic responsiveness of the direct pathway. Collectively, these receptor changes support modulation of striatal dopaminergic signaling, although the associated changes in D1-spiny and D2-spiny projection neuron activity will require direct functional examination.

Proteomic analysis of the control striatum provided additional insight into the fundamental molecular response to TI stimulation. Among the high-confidence proteins, KCNA3 showed marked downregulation, whereas CACNG4 and PTK2B were increased. KCNA3 encodes the voltage-gated potassium channel Kv1.3, which participates in the regulation of membrane excitability and cellular signaling. Previous studies have linked Kv1.3 inhibition to altered immune-cell activity and improved conduction in demyelinated axons, although the direct relevance of these observations to striatal neurons remains to be established (Singh et al., 2020). In comparison, CACNG4 and PTK2B provide more direct links to neuronal and postsynaptic regulation. CACNG4, also known as TARP γ -4, is an auxiliary

AMPA receptor subunit that regulates receptor trafficking, channel properties, and postsynaptic currents(Radin et al., 2018). PTK2B, or Pyk2, is a calcium-sensitive kinase involved in activity-dependent neuronal signaling and glutamate receptor-associated plasticity(De Luca et al., 2025a). Consistent with these proteins, GO enrichment implicated cytoskeletal regulation, distal axonal components, and neuronal GTPase-related functions. These findings suggest that TI stimulation in the healthy striatum engages a broader program involving neuronal excitability, receptor regulation, intracellular signaling, and structural remodeling.

The proteomic response of the MPTP-treated striatum differed from that observed in control mice. Among the 156 candidate TI-responsive proteins, HECA and TRAF3IP3 were increased and NCF1 was decreased after TI stimulation. NCF1 is a regulatory component of the NADPH oxidase complex and contributes to reactive oxygen species generation in immune and neural cells(Keeney et al., 2024). Its reduction therefore raises the possibility that TI modulates oxidative or inflammatory signaling in the parkinsonian striatum. However, the functional consequences of NCF1 downregulation should be confirmed through targeted assessment of NADPH oxidase activity and oxidative stress. Additional candidate proteins, including UNC79, GRIA3, NETO2, CAMK2G, CREB1, TANC1, and ERBIN, further suggested effects on neuronal excitability, glutamatergic transmission, calcium-dependent signaling, and postsynaptic organization(Piard et al., 2020; Sasazawa et al., 2022).

More importantly, preranked GSEA incorporating all quantified proteins identified postsynaptic density as the most robust pathway-level signal associated with TI stimulation in MPTP-treated mice. The postsynaptic density gene set was significantly enriched toward the MPTP-TI group after Benjamini–Hochberg correction. Its leading-edge subset included DLG4/PSD95, GRIA1, GRIA2, GRIN1, GRIN2A, GRIN2B, SHANK-family proteins, and CAMK2 isoforms. Thus, the enrichment was not attributable to a single highly altered protein but instead reflected coordinated changes across glutamate receptors, scaffold proteins, calcium-sensitive kinases, and other components of the postsynaptic signaling complex. Translation at the postsynapse also showed a positive enrichment trend, although it did not remain significant after correction for multiple comparisons. This finding may suggest altered postsynaptic protein synthesis or turnover.

The targeted biochemical measurements provided convergent support for the proteomic evidence of postsynaptic protein-network remodeling. MPTP treatment increased GluN2B, GluR1, and GluR2 protein abundance while reducing PSD95 expression. Increased GluN2B and GluR1 levels are consistent with altered glutamatergic signaling in the dopamine-depleted striatum. Although GluR1-containing AMPA receptors contribute to activity-dependent synaptic strengthening, excessive receptor abundance or abnormal membrane recruitment may also contribute to dysregulated excitatory transmission(Chang et al., 2019). The concurrent increase in GluR2 may represent a compensatory response favoring calcium-impermeable AMPA receptors and limiting excessive calcium entry. TI stimulation attenuated the MPTP-associated changes in GluN2B, GluR1, and GluR2 and partially restored PSD95 expression, whereas GluN2A remained unchanged. The absence of a uniform effect across all receptor subunits suggests

that TI did not simply suppress or enhance glutamatergic signaling but instead modified the molecular composition of the postsynaptic apparatus.

PSD95 is a major scaffold protein within the postsynaptic density and regulates the localization, clustering, and signaling of NMDA and AMPA receptors(De Luca et al., 2025b). Its reduction after MPTP treatment is consistent with disruption of postsynaptic molecular organization, whereas its partial recovery after TI stimulation suggests reorganization of the scaffold supporting excitatory receptor signaling. The agreement between PSD95 Western blotting and the postsynaptic density GSEA result strengthens the interpretation that TI affects a broader postsynaptic protein network rather than isolated receptor subunits. Such coordinated remodeling may contribute to improved corticostriatal information processing and motor performance. Nevertheless, the present findings are based on protein abundance and pathway enrichment and do not directly demonstrate restoration of synaptic transmission or activity-dependent plasticity. Measurements of receptor membrane localization, synaptic currents, and LTP or LTD will be required to determine the functional consequences of these molecular changes.

Several limitations should be acknowledged. The subacute MPTP model reproduces nigrostriatal dopaminergic injury and motor dysfunction but does not encompass the full chronic and progressive pathology of human PD. Molecular measurements were obtained from bulk striatal tissue and therefore cannot distinguish responses among D1-spiny projection neurons, D2-spiny projection neurons, interneurons, and glial cells. In addition, the control and MPTP proteomic comparisons were acquired as independent experiments, preventing formal disease-by-stimulation interaction analysis. Finally, functional electrophysiological measurements were not performed. Nevertheless, the convergence of behavioral, histological, biochemical, and proteomic evidence supports a consistent association between striatal TI stimulation, motor recovery, dopaminergic regulation, and postsynaptic network remodeling.

In conclusion, repeated striatal TI stimulation ameliorated multiple motor deficits in MPTP-treated mice and attenuated MPTP-associated abnormalities in the nigrostriatal dopaminergic system. TI increased DA receptor expression, normalized selected NMDA and AMPA receptor subunits, partially restored PSD95, and induced coordinated enrichment of postsynaptic density-related proteins. Together with previous clinical findings from our group demonstrating acute motor benefits after GPi- and STN-targeted TI stimulation in patients with PD, the present results provide a translational bridge between clinical efficacy and underlying molecular responses. These findings support striatal dopaminergic and postsynaptic protein-network remodeling as potential contributors to TI-mediated motor improvement and provide a foundation for the development of target- and parameter-specific TI interventions for PD.

4. Materials and Methods

4.1 Animals and PD models

All procedures were approved by the Ethics Committee of Shanghai University of Sport and were performed in accordance with institutional guidelines and the ARRIVE recommendations. Ethics Approval Number is 102772025DW020. Male C57BL/6 mice aged 7 weeks were housed under a 12-h light-dark cycle at $22 \pm 2^\circ\text{C}$ and $60\% \pm 10\%$ humidity, with food and water available ad libitum. Forty-eight mice were randomly assigned to four groups ($n = 12$ per group): Con-Sham, Con-TI, MPTP-Sham, and MPTP-TI. The number of animals used for each behavioral, histological, biochemical, and proteomic analysis is provided in the corresponding figure legend.

4.2 MPTP model

MPTP hydrochloride (MedChem Express LLC, Princeton, NJ, USA) was administered intraperitoneally for 10 consecutive days. The initial dose was 30 mg/kg (Zhou et al., 2019). Control mice received equivalent volumes of saline. Model establishment was assessed by motor testing and TH immunohistochemistry before the TI intervention.

4.3 Electrode placement and TI stimulation

The mice were placed in an isoflurane induction cassette to anesthetize the mice, and the mice were numbed for approximately 10s. The mice scalp is disinfected with iodine and 70% ethanol, and the scalp is cut open with scissors and disinfected with alcohol. The mice were placed on the skull locator, erythromycin eye ointment was applied to protect the eyes, the skin was cut open to expose the skull, the location of Bregma (fontanelle point) was located and marked, and the coordinates of the screw electrodes were determined. The coordinates of the electrode positions in the striatum region for TI stimulation are shown in Fig. 1b. Electrode 1: AP: -2.75 mm, ML: -1.5 mm. Electrode 2: AP: 0.25 mm, ML: -1.5 mm. I1 and I2 electrodes are paired with a 10mm diameter electrode, connected to the same side of the cheek. The schematic diagram of the TI stimulated mice shows I1 connected at 2000 Hz with a current of 0.15 mA and I2 connected at 2020 Hz with a current of 0.15 mA, forming an electrode circuit with the electrodes on the same side of the skull respectively, creating an envelope wave with a Δf of 20 Hz. TI stimulation was administered daily for 10 minutes under isoflurane anesthesia over 7 consecutive days.

4.4 Behavioral testing

4.4.1 Beam balance test

The beam balance test is constructed to evaluate motor coordination and balance deficits. To assess severity of motor impairment, recording the time it takes for the mouse to cross the balance beam (1 cm wide and 50 cm long) and number of times the feet slipped. Each mouse was tested three times. A stopwatch was used to record the cross time (Ding et al., 2023).

4.4.2 Gait test

The Gait test was utilized to quantify gait abnormalities, including stride rate and hindlimb stride width (Agrawal & Mishra, 2019). Mice were acclimated to traversing a narrow, enclosed runway (42 cm

length × 29.7 cm width) lined with white paper and leading to a darkened goal box. On the day of testing, the hind paws were coated with a non-toxic black ink, and each mouse was allowed to walk down the runway. The resulting footprint patterns were then analyzed to measure the stride rate (ratio of runway distance to time) and the stride width (lateral distance between left and right hind paws) from a minimum of three sequential steps.

4.4.3 Pole test

The pole test is a well-established behavioral assay for assessing motor coordination and bradykinesia in rodent models(Zhou et al., 2019). Prior to testing, mice were acclimated to the apparatus through a pre-training session. During the formal test, each mouse was placed head-up at the top of a vertical, rough-surfaced pole (50 cm in height, 1 mm in diameter). The latency to descend to the base of the pole was recorded. Each mouse underwent three trials, and the average latency from the three trials was used for statistical analysis.

4.4.4 Wire Suspension Test

The wire suspension test was employed to assess forelimb grip strength, motor coordination, and endurance in mice(Liu et al., 2023). Each mouse was suspended by its forepaws on the midpoint of a horizontal steel wire (50 cm in length, positioned 25 cm above a soft-landing surface). Performance was evaluated using a graded scoring system (0, fell off immediately; 1, maintained grip using two forepaws only for the trial duration; 2, gripped the wire with both forepaws and attempted to climb upward; 3, gripped the wire with both forepaws and one or both hindpaws; 4, gripped with all four paws and tail wrapped around the wire; and 5, successfully escaped to either supporting platform). Additionally, the latency to fall (or escape) was recorded for each trial, with a maximum cutoff time of 30 seconds.

4.5 Proteomic analysis

Striatal tissue was analyzed using 4D-DIA relative quantitative proteomics (Gene DENOVO). Protein concentration was determined using the BCA assay, proteins were enzymatically digested, and peptides were analyzed by liquid chromatography-tandem mass spectrometry. Spectra were searched against a mouse reference protein database, and protein-level intensities were normalized within each analytical experiment. Differential analysis was conducted using limma(Wu et al., 2021). The control comparison (Con-Sham vs Con-TI) and MPTP comparison (MPTP-Sham vs MPTP-TI) were acquired in separate analytical batches. Consequently, normalization, differential testing, and enrichment analysis were performed independently within each batch. Candidate TI-responsive proteins were defined as proteins with an absolute fold change ≥ 1.2 or $\leq 1/1.2$ with $p\text{-value} \leq 0.05$. Proteins that also met a Benjamini-Hochberg adjusted $P < 0.05$ were considered high-confidence differentially expressed proteins. Volcano plots were used to distinguish high-confidence proteins from nominal candidate proteins. GO over-representation analysis was performed separately for each batch using the proteins quantified in that batch as the background set. GSEA was performed for the MPTP proteomic dataset using all quantified proteins rather than only threshold-defined candidates. Protein entries were mapped to gene symbols, and duplicate symbols were resolved by retaining the entry with the largest absolute ranking score.

GSEA was performed on the MPTP proteomic dataset using all quantified proteins rather than only proteins meeting the predefined differential-expression thresholds. Protein entries were first mapped to their corresponding gene symbols. When multiple protein entries mapped to the same gene symbol, the entry with the largest absolute ranking score was retained. The resulting unique gene symbols were ranked using a composite statistic defined as $\text{sign}(\log_2 \text{fold change}) \times -\log_{10}(P \text{ value})$, such that positive scores represented higher protein abundance in the MPTP-TI group and negative scores represented higher abundance in the MPTP-Sham group. GO biological process, cellular component, and molecular function terms were used as gene sets. GSEA was conducted using the clusterProfiler package with the fgsea algorithm, a minimum gene set size of 15, a maximum gene set size of 500, and the Benjamini–Hochberg method for multiple-testing correction. Positive normalized enrichment scores (NES) indicated enrichment toward the MPTP-TI group, whereas negative NES values indicated enrichment toward the MPTP-Sham group. Terms with BH-adjusted P values < 0.05 were considered statistically significant, whereas terms with nominal P values < 0.05 that did not remain significant after BH correction were interpreted as exploratory findings.

4.6 Western blotting

Total proteins were extracted from the stimulated striatum of all groups sacrificed 24 h after the last stimulation by using ice cold RIPA buffer. The protein concentrations were assayed by using a BCA protein assay kit (Beyotime Biotechnology, Shanghai, China). Protein lysates (30 μg) were loaded onto 10% or 12% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene difluoride (PVDF) membranes. The membranes were blocked for 1.5 h by using 5% non-fat dry milk blocking buffer. Then, the blots were incubated overnight at 4°C with primary antibodies, including rabbit anti-TH (58844S, Cell Signaling Technology, 1:2000), rabbit anti-DRD1 (17934-1-AP, Proteintech, 1:1000), rabbit anti-DRD2 (55084-1-AP, Proteintech, 1:1000), rabbit anti-NMDAR2A (83465-2-RR, Proteintech, 1:1000), rabbit anti-NMDAR2B (21920-1-AP, Proteintech, 1:1000), rabbit anti-GluR1 (67642-1-Ig, Proteintech, 1:1000), rabbit anti-GluR2 (11994-1-AP, Proteintech, 1:1000), rabbit anti-PSD95 (20665-1-AP, Proteintech, 1:1000). The next day, after washing thrice for 5 min in phosphate-buffered saline and 0.1% Tween 20 (TBST), membranes were incubated for 2h at room temperature with anti-rabbit IgG horseradish peroxidase (HRP)-conjugated secondary antibodies (7074S, Cell Signaling Technology, 1:2500). After washing thrice, the proteins were visualized with automatic chemiluminescence apparatus (Tanon Biotechnology, Shanghai, China) and quantified by densitometry.

4.7 Immunofluorescence and immunohistochemistry

For immunohistochemistry staining, the brain was removed and dehydrated in 20% and 30% sucrose solution, then cut into sections at a thickness of 20 μm , rinsed in PBS, and incubated in 3% H_2O_2 to quench endogenous peroxidase activity. After washing with PBS, sections were incubated in 10% goat serum followed by 0.3% TritonX-100 in PBS, then subsequently by overnight incubation at 4°C with primary antibodies, including, anti-TH antibody (58844S, Cell Signaling Technology, 1:500), anti-DRD1 antibody (17934-1-AP, Proteintech, 1:500), and anti-DRD2 antibody (55084-1-AP, Proteintech, 1:500) in a humidified chamber. After washing with PBS, sections were incubated with secondary antibodies at 37

°C ovens for 80 min. Quantitative analysis of the total number of TH positive neurons in the SNpc was conducted. All images were viewed and captured with a digital camera (Olympus, Tokyo, Japan). Immunofluorescence staining followed a protocol like that of immunohistochemistry. Sections were incubated with primary anti-c-fos (2250S, Cell Signaling Technology, 1:500) overnight at 4°C. After removal of the primary antibody, a fluorochrome-conjugated secondary antibody (FITC) was applied and incubated at 37°C for 1 hour. Nuclei were counterstained with DAPI, and the stained sections were visualized under a Confocal laser scanning microscope (Nikon, Tokyo, Japan).

4.8 Statistical analysis

The data were processed using the SPSS 26.0 software. All data were presented as mean $\pm$ standard error of the mean (SEM). Statistical analysis was performed using two-way analysis of variance (ANOVA). The level of statistical significance was set at $P < 0.05$.

Declarations

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Conflicts of Interest

All authors declare that there are no conflicts of interest regarding the publication of this article.

Author contributions

Ziyu Wang: Conceptualization, original draft, Visualization

Jin Peng: review and editing, Visualization

Yu Liu: Supervision: Methodology, Funding acquisition,

Xiaohui Wang: Supervision: Methodology

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Figures

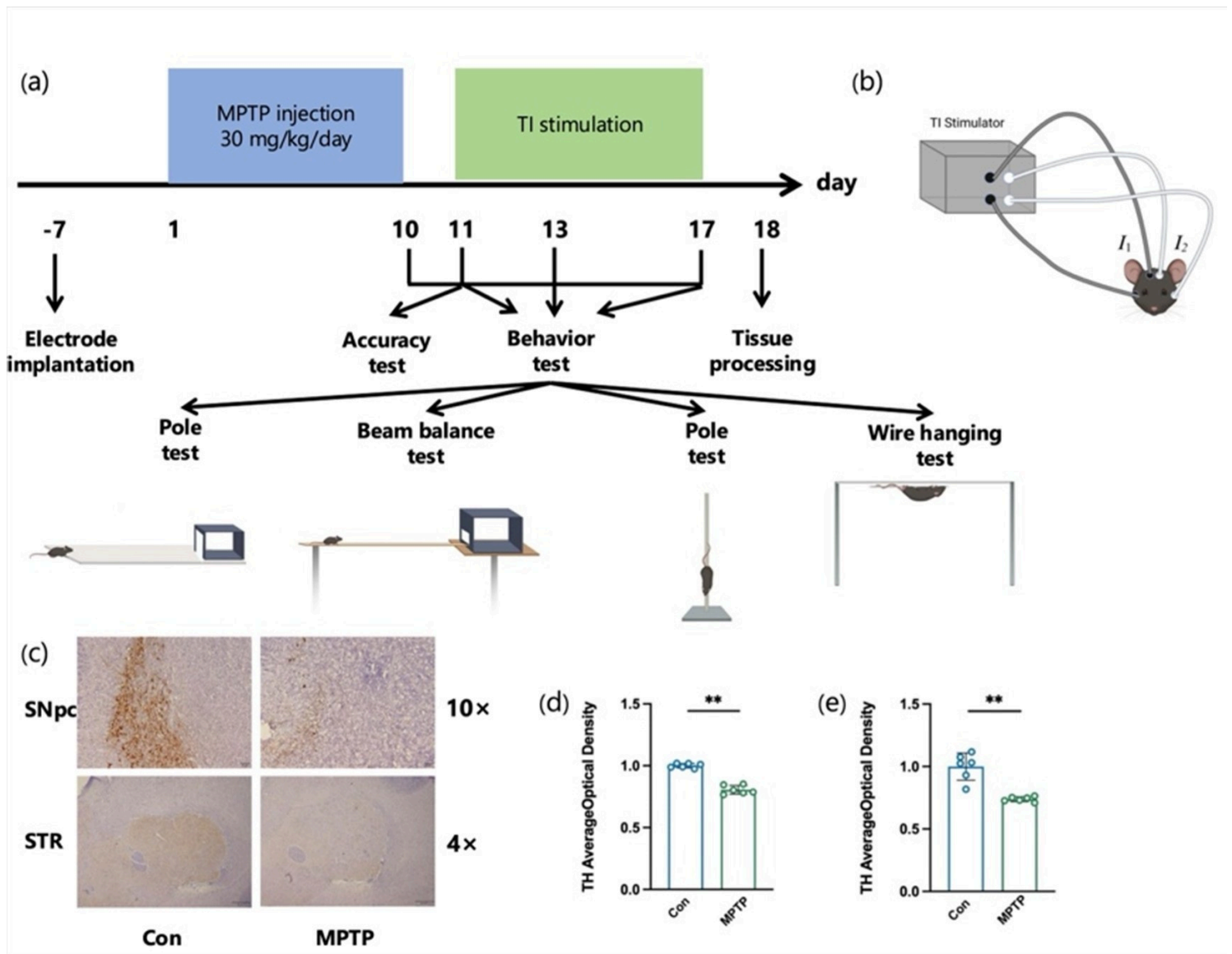


Figure 1

Experimental design and establishment of the MPTP model

(a) Experimental design; (b) TI stimulation mode diagram; (c) Immunohistochemistry staining of TH in the SNpc (10x) and striatum (4x) in Con group and MPTP group; (d) A significant reduction in TH⁺ neurons was observed in the SNpc ($n=6$, $P < 0.01$); (e) A significant reduction in TH⁺ neurons was observed in the striatum ($n=6$, $P < 0.01$);

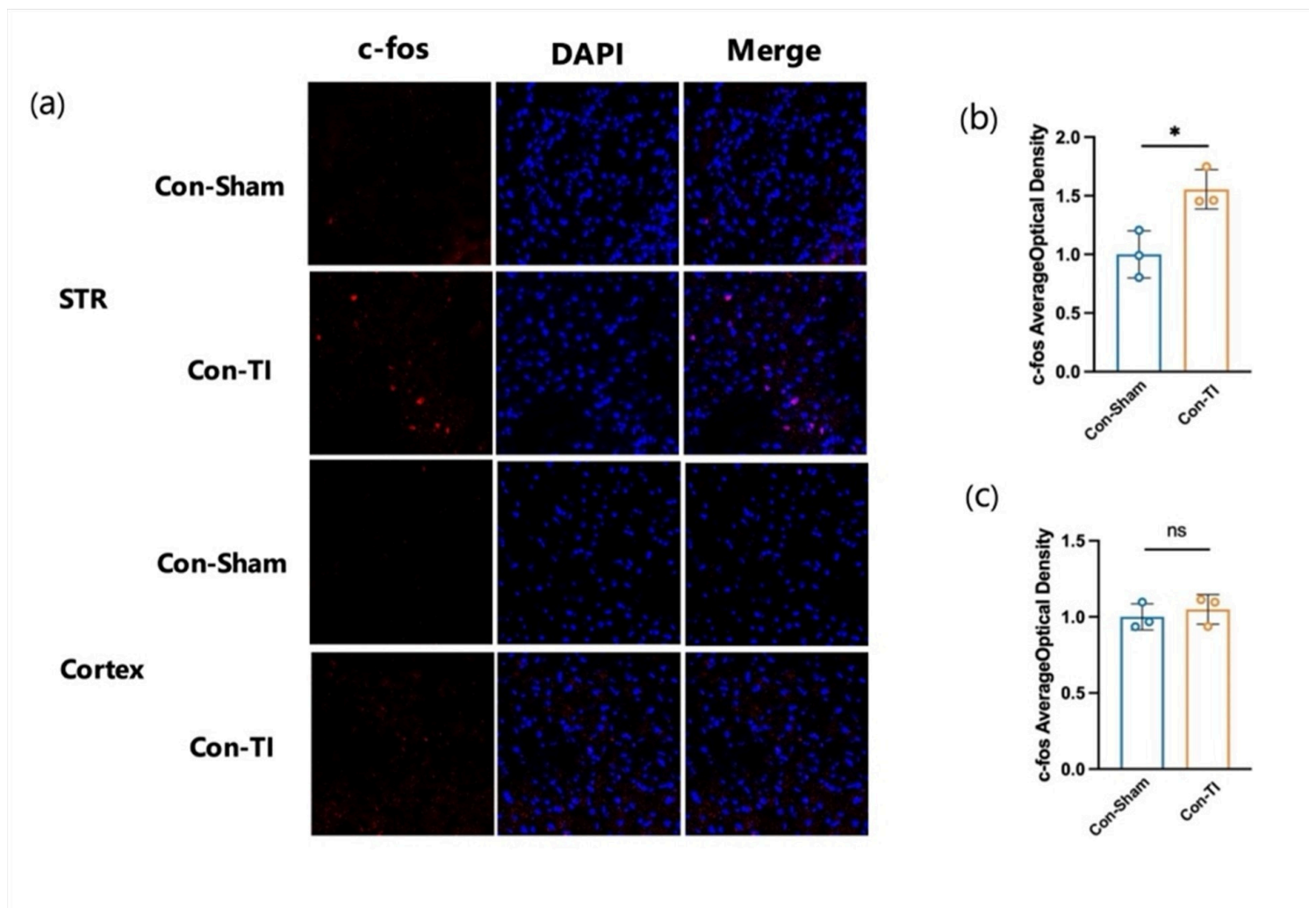


Figure 2

The accuracy of TI stimulation.

(a) Assessment of TI Stimulation Precision via c-Fos Immunofluorescence; (b) TI stimulation induced striatal c-Fos expression in the Con-TI group but not in the Con-Sham group ($n=6$, $P < 0.05$). (c) TI stimulation did not cause significant difference in c-fos expression in the cortical regions between the two groups.

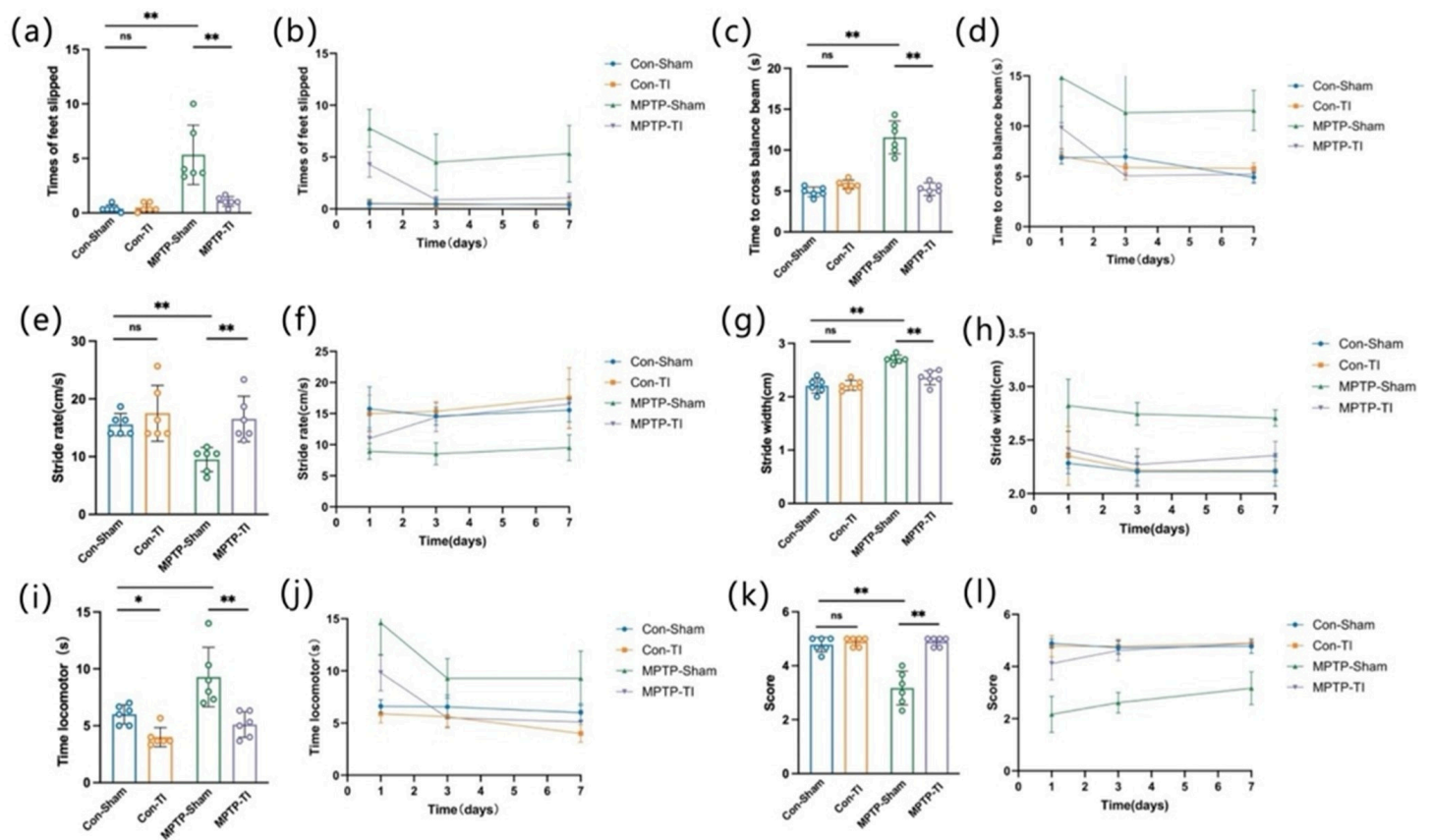


Figure 3

Striatal TI stimulation improves motor performance in MPTP-treated mice.

(a, b) Number of foot slips in the beam balance test on day 7 and across days 1, 3, and 7 of stimulation, respectively. (c, d) Time required to traverse the balance beam on day 7 and across days 1, 3, and 7, respectively. (e, f) Stride rate during Gait test on day 7 and across days 1, 3, and 7, respectively. (g, h) Stride width on day 7 and across days 1, 3, and 7, respectively. (i, j) Pole descent time on day 7 and across days 1, 3, and 7, respectively. (k, l) Wire suspension scores on day 7 and across days 1, 3, and 7, respectively. MPTP-Sham mice displayed impaired motor coordination, gait, bradykinesia-related performance, and suspension ability compared with Con-Sham mice, whereas TI stimulation attenuated these deficits in MPTP-treated mice ($n=6$, * $P < 0.05$, ** $P < 0.01$).

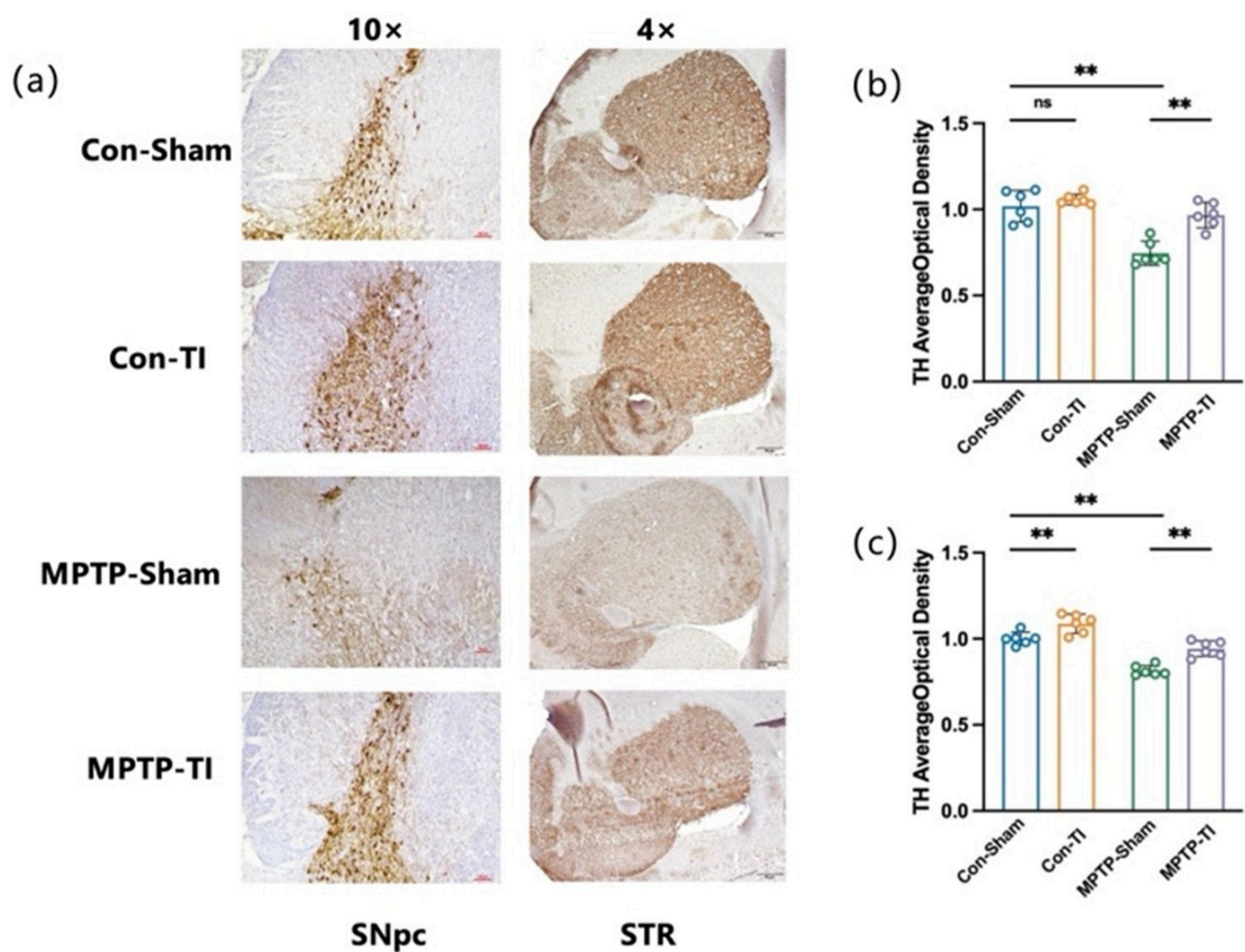


Figure 4

TI stimulation attenuates MPTP-associated reductions in nigrostriatal TH immunoreactivity.

(a) Immunohistochemical staining for TH positive neurons in the left SNpc (10x) and STR (4x). (b)(c) The number of TH positive neurons in the left SNpc and striatum is significantly decreased in MPTP-Sham group compared with Con-Sham group and significantly increased in MPTP-TI group ($n=6$, $^*P < 0.05$, $^{**}P < 0.01$).

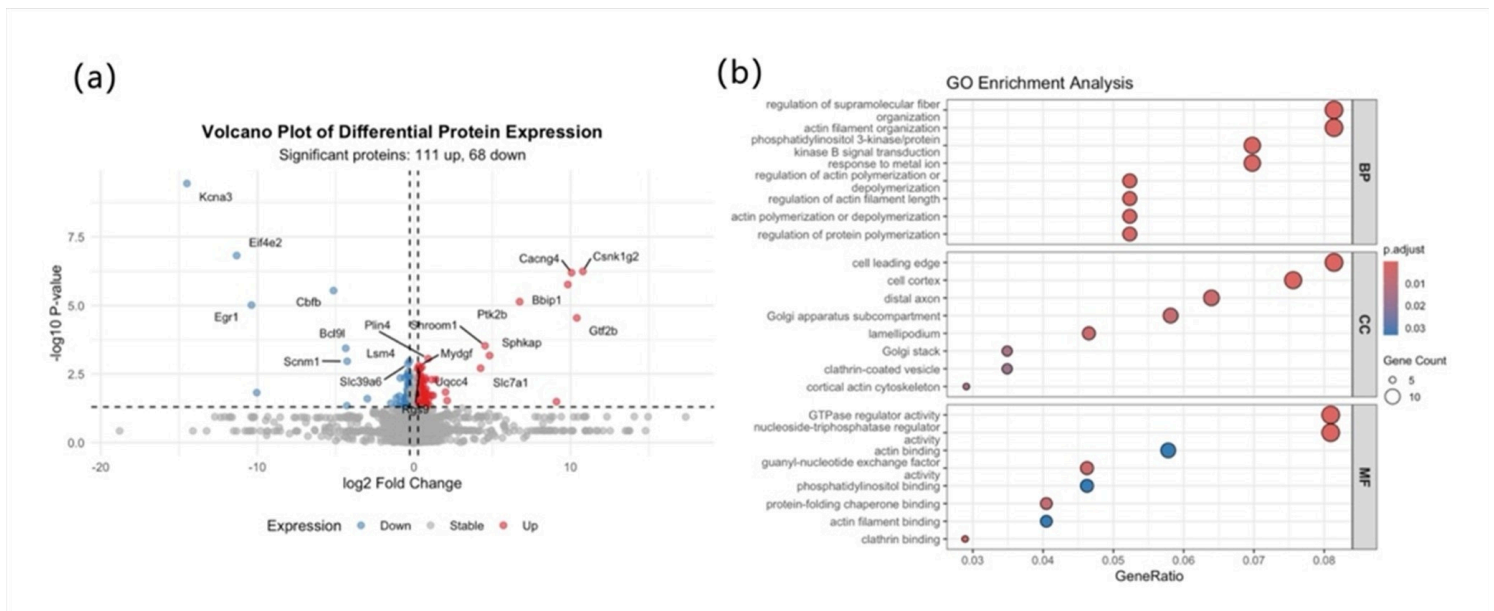


Figure 5

Proteomic remodeling induced by TI stimulation in the control striatum

(a) Volcano plot of differential protein expression between Con-Sham and Con-TI. The red dots indicate upregulated genes, and the blue dots indicate downregulated gene; (b) Gene Ontology (GO) enrichment analysis of differential protein expression in biological process (BP), Cellular Component (CC) and molecular function (MF).

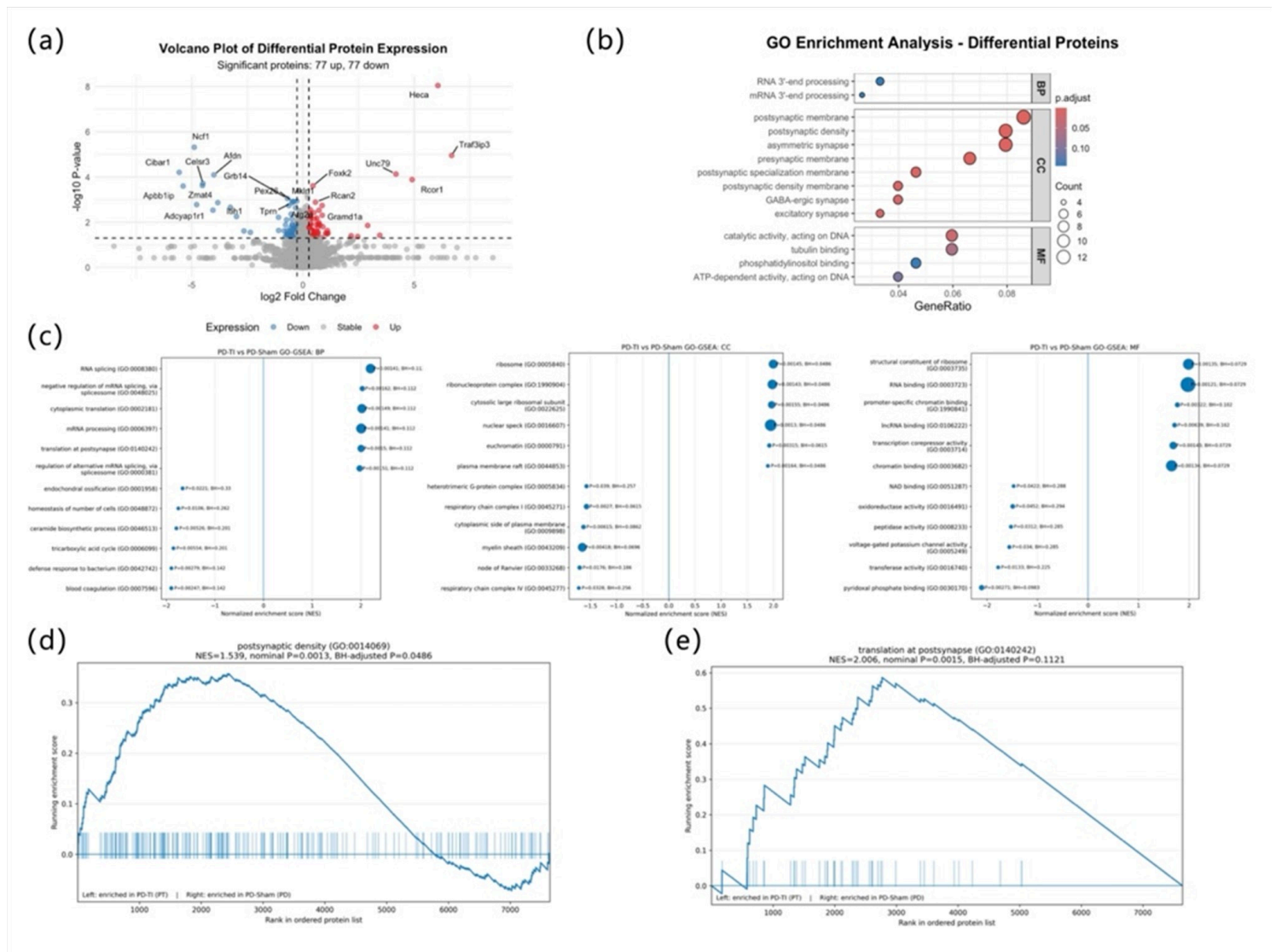


Figure 6

Proteomic and GSEA analyses reveal postsynaptic remodeling after TI stimulation in MPTP-treated mice

(a) Volcano plot of differential protein expression between MPTP-Sham and MPTP-TI. (b) Gene Ontology (GO) enrichment analysis of differential protein expression in biological process (BP), Cellular Component (CC) and molecular function (MF). (c) Summary of preranked GSEA results for GO BP, CC, and MF terms using all 7,620 unique protein-associated gene symbols quantified in the MPTP proteomic dataset. Positive normalized enrichment scores (NES) indicate enrichment toward the MPTP-TI group, whereas negative NES values indicate enrichment toward the MPTP-Sham group. (d) GSEA enrichment plot for the postsynaptic density gene set, which was significantly enriched toward the MPTP-TI group (NES = 1.539, nominal $P = 0.00129$, BH-adjusted $P = 0.0486$).

(e) GSEA enrichment plot for translation at the postsynapse, which showed positive enrichment toward the MPTP-TI group (NES = 2.006, nominal $P = 0.00150$) but did not remain significant after BH correction (adjusted $P = 0.112$). The vertical ticks in the enrichment plots indicate the positions of gene-set members in the ranked protein list, and the running enrichment score indicates their cumulative

distribution across the ranking. Collectively, these analyses support coordinated remodeling of postsynaptic protein networks following TI stimulation in MPTP-treated mice.

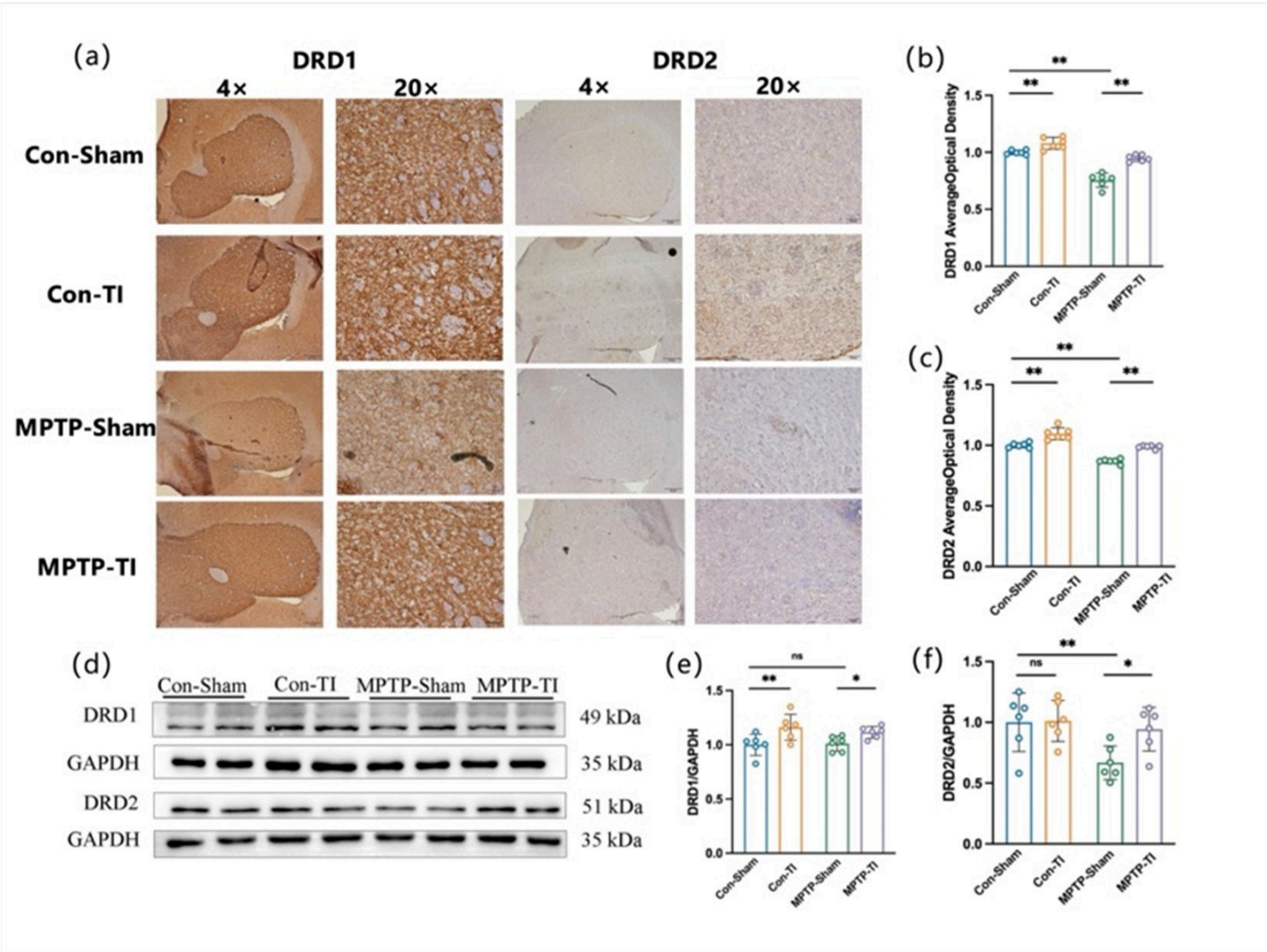


Figure 7

TI stimulation modulates striatal dopamine receptor expression.

(a) Immunohistochemical staining for DRD1 and DRD2 in the STR; (b)(c) The expression levels of both DRD1 and DRD2 in the STR were significantly decreased in the MPTP-Sham group compared with the Con-Sham group, while TI stimulation significantly elevated their expression in the MPTP-TI group and Con-TI group; (d) Western Bolt analysis for DRD1 and DRD2 in the STR; (e) No significant difference was observed between the Con-Sham and MPTP-Sham groups following MPTP injection, whereas TI stimulation significantly enhanced this parameter in both the Con-TI and MPTP-TI groups; (f) The expression of DRD2 was significantly reduced in the MPTP-Sham group compared to the Con-Sham group, whereas TI stimulation significantly increased DRD2 levels in both the Con-TI and MPTP-TI groups (n=6, *P < 0.05, **P < 0.01)

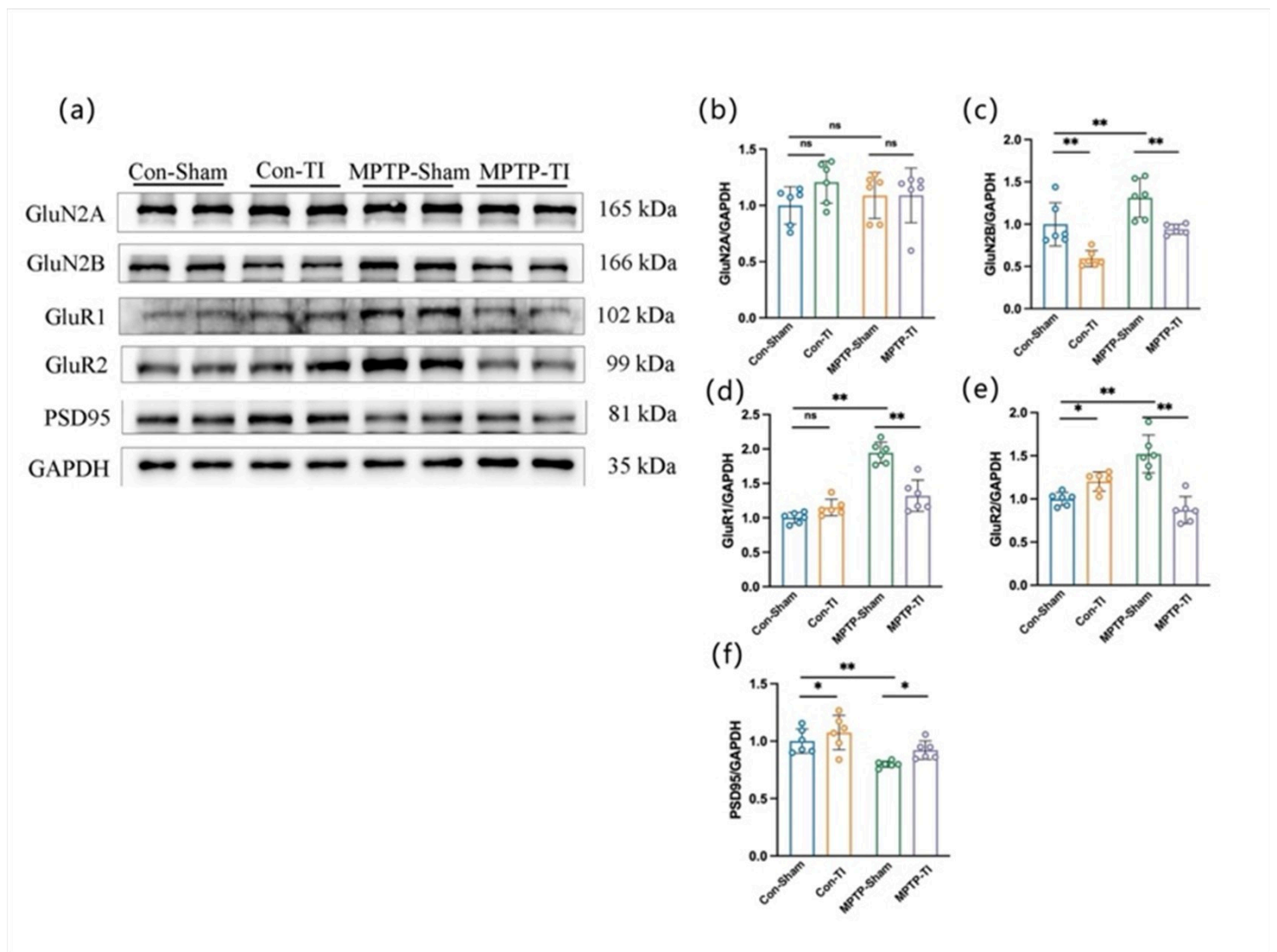


Figure 8

TI stimulation normalizes glutamate receptor-associated protein abnormalities and partially restores PSD95.

(a) Western Blot analysis of synaptic plasticity related proteins; (b) No significant difference in GluN2A protein levels was observed across the four experimental groups; (c)(d)(e) The level of GluN2B, GluR1 and GluR2 was significantly increased in the MPTP-Sham group compared to the Con-Sham group, whereas TI stimulation rescued this elevation in the MPTP-TI group; (f) The level of PSD95 was significantly reduced in the MPTP-Sham group compared to the Con-Sham group, whereas TI stimulation rescued this loss in the MPTP-TI group (n=6, * $P < 0.05$, ** $P < 0.01$)

Supplementary Files

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