Striatal Syntaixin 1A Plays a Protective Role Against Iminodipropionitrile Induced Tic Disorder Through Interaction with Dopamine Transporter

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Research Article

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Abstract

An important mechanism of Tic disorder (TD) is dysfunction in the dopamine (DA) system. Our pilot observation found the expression of Syntaxin 1A (STX1A), a presynaptic SNARE complex, changed in the striatum of TD animals. The present study aimed to clarify the biological role of striatal STX1A in the pathological state of TD and the specific mechanism of its regulation of the dopaminergic system. The TD rat model was established using iminodipropionitrile (IDPN). Adenovirus was used to modulate the expression of STX1A and dopamine transporter (DAT) in vivo and vitro. Primary culture of striatal dopaminergic neurons was performed for in-vitro observation of the DA reuptake, CO-IP analysis of the interaction between STX1A and DAT. First, using immunofluorescence staining, Western blotting, and qPCR, we found that the IDPN induced TD model had reduced striatal STX1A expression. In vitro, the DA content in the supernatant was significantly lower in the STX1A overexpressed group, and the intracellular DA content was significantly higher. Overexpression of STX1A in vivo partially counteracts the IDPN-induced TD-like behaviors, including bite time and head shaking time. Meanwhile, in-vivo knockdown of STX1A can aggravates TD-like behaviors. Further, DAT was overexpressed in vivo, and the TD-like behavior was alleviated. Interestingly, overexpression of DAT in the striatum resulted in increased levels of STX1A. In order to clarify the interaction between DAT and STX1A, the CO-IP analysis was conducted based on the protein of purified striatal dopaminergic neurons. Compared to the IgG control, the blots of DAT and STX1A showed significant binding of each other. Striatal STX1A expression is decreased in TD development, and STX1A plays an anti-TD role possibly through interaction with DAT, which maintains the DA reuptake. The exorbitant DA signal caused by STX1A inhibition drives the pathological stereotyped behavior.

Introduction

Tic disorder (TD) is a neurodevelopmental movement disorder characterized by multiple motor and vocal tics [1]. According to the development process, it can be divided into transient TD, chronic TD and Tourette syndrome (Tourette syndrome, TS), among which TS is the most typical type. TD affects at least 1% of the population worldwide, and the incidence is still rising [2]. TD is associated with attention deficit hyperactivity disorder, obsessive-compulsive disorder, and emotional disorder, which may severely influence children's social adaptation ability [3–6]. Moreover, it can lead to lifelong illnesses. Currently, common treatments include psycho-behavioral therapy and drug therapy; and symptomatic treatment was majorly applied to alleviate patients' tic symptoms, but it cannot fundamentally improve the prognosis.

Overall, this chronic neurobehavioral disorder has unclear pathophysiology. One of the important mechanisms is the dysfunction in the dopamine (DA) system, in particular the striatal dopaminergic neurons or striatal dopamine signal system [7–11]. The relationship between dopaminergic neurons and TD has been reported by different teams. Years of research and clinical practice have proved DA receptor antagonists to be effective agents in the treatment of TS or TD (allowing a significant tic reduction of about 70%) [12]. Central DA alterations and dysfunction of the dopaminergic system is an important

cause of TD, dopamine D2 receptor is noticed to be associated with the occurrence of TD [13–15]; dopaminergic neurons in the striatum may be an essential factor that affects the occurrence of TD [16]. Besides, changes in the structure and function of the striatum were observed under the pathological state of TD [17–20]. Moreover, imaging studies suggested that TS individuals have presynaptic and post-synaptic striatum dopamine neuron dysfunctions [21]; PET technology showed that in TS patients the ventral striatum dopamine release is increased [22]. Collectively, dopaminergic signaling impacts TD-like behaviors and pathological changes, striatal dopaminergic neurons are closely related to the pathogenesis and treatment of TD. But the direct evidence is not sufficient, and the detailed upstream mechanisms are still unclear. The presynaptic dopamine transporter (DAT) is an important factor that modulates the dopaminergic signaling, which recaptures released DA, thereby limiting synaptic DA availability, and maintaining dopaminergic tone. Through DAT, released DA is re-uptaken into nerve terminals, and this is a crucial mechanism of steady-state balance for dopaminergic signaling. For TD treatment, DAT is a valuable exploration target.

To unravel mechanism of TD, a variety of animal models have been developed. Establishment of TD model using iminodipropionitrile (IDPN) is a simple, easy-to-use, and widely applied method [23]. In this study, we established a TD rat model through a short-to-medium administration of IDPN. In this model, neuropsychiatric disorder is developed, characterized by stereotyped repetitive involuntary tics, pedaling, biting, head twitching, shaking claws, continuous rotation, etc. [24–26].

In addition, our pilot observation found that the expression of Syntaxin 1A (STX1A), a presynaptic SNARE complex, changed in the striatum of the TD model. The present study aimed to clarify the striatal STX1A alteration in the pathological state of TD, its biological effects on the occurrence of TD, and the specific mechanism of its regulation of the dopaminergic system.

Materials And Methods

Animals and the TD model

The SPF-grade SD rats (male, 6 weeks old, weight 200±10 g) were purchased from Changzhou Cavens Experimental Animal Co., Ltd. All experimental animals were adapted to feed for a week before the formal experiment. First, we conducted a pilot experiment to validate the IDPN induced TD model. Rats in the TD group were intraperitoneally injected with iminodipropionitrile (IDPN, 200 mg/kg, Sigma Chemical Co., St. Louis, MO, USA, at 9:00-11:00, for 7 consecutive days). The control group was injected with the same dose of saline (5 ml/kg/day). In our previous investigation, the stereotypic behavior was triggered by IDPN injection (including biting, head twitching, shaking claws, continuous rotation, etc., and the stereotypic behavior score was significantly elevated).

For each batch of animals, after the behavioral observation, animals were sacrificed, and brains were sampled. A part of samples was fixed in 4% paraformaldehyde; for the other part of samples, the striatum tissue was separated for each animal and homogenized, the proteins and RNAs were extracted and stored at -80℃.

Primary culture of striatal dopaminergic neurons

Two SPF-grade SD pregnant mice were purchased from the Changzhou Cavens Laboratory Animal Co., Ltd., and at least 10 pups were obtained for primary culture of dopaminergic neurons. The 3-to-5-day-old SD rats were soaked in 75% ethanol for 30 min. Each rat was transferred to an ultra-clean table; under the aseptic conditions, the brain tissue was taken out, rinsed with pre-cooled PBS, and placed in a petri dish. Under a microscope, the striatal nucleus was exposed. The striatum was picked off and placed in a sterile EP tube, and PBS was added to rinse the tissue. Next, 0.25% pancreatin was added. After a 10-min digestion, the tissue was pipetted and transferred to a new EP tube. An appropriate amount of H-DMEM complete medium (90% H-DMEM medium + 10% FBS + 1% penicillin/streptomycin) was added, the tissue suspension was pipetted and centrifuged (1000 rpm for 5 min). The supernatant was removed, an appropriate amount of Neurobasal complete medium (90% Neurobasal medium + 10% FBS + 1% penicillin/streptomycin) was added and the single-cell suspension was generated by pipetting. All cells then passed through a 70-μm cell sieve. The filtered suspension was added to the cell culture flask, an appropriate amount of Neurobasal complete medium was supplemented. The plate was placed in a culture incubator (37°C, 5% CO_2). One day after the cells were extracted, the medium was changed. Then, the medium was refreshed every 3 days. The striatal dopaminergic neurons were identified by the tyrosine hydroxylase (TH) staining.

Realtime qPCR

Total RNA was extracted from the striatum region of the brain using TRIZOL (Takara, 9108), and cDNAs were synthesized using a TAKARA PrimeScript™ RT reagent Kit (Takara, RR047A). PCR amplification reactions were conducted using a SYBR Green Supermix system (Takara, RR820A) in a 20 μl reaction containing 0.8 μl primers (0.4 μl of the 10 μM Forward primer and 0.4 μl of the 10 μM Reverse primer, respectively) and 2 μl cDNA. The cycling program was as follow: (1) denaturation at 95°C for 1 minute, (2) 40 cycles of (95°C for 5 s + 55°C for 30 s + 72°C for 30 s), (3) melting curve. All measurements were performed in triplicates. The primers pairs (forward and reverse, respectively) used in amplification were as follow.

β-actin: Forward GGGCTTTATTGACAAGATTGCT; Reverse TCGGTAGTCTGACTGAGTGGC.

STX1A: Forward ACCACAGCTGAGAGGGAAATCG; Reverse AGAGGTCTTTACGGATGTCAACG.

ELISA

For each well, the cell supernatant was collected and centrifuged at 1000×g for 20 min. Then, the supernatant was stored at 2 to 8°C. The above samples were used to detect the concentration of dopamine in the cell culture supernatant. In addition, the cells in each well were gently washed with pre-cooled PBS for 3 times, 200 μl of lysis buffer was added to each well, cells were lysed by pipetting and the lysis solution centrifuged at 10000×g for 5 min. Next, this supernatant was also stored at 2 to 8°C. The above samples were used to detect the concentration of intracellular dopamine. The DA ELISA Kit

(Wuhan Fine Biotech Co., Ltd, Product code EU0392) was used for measurement of the supernatant dopamine content and intracellular dopamine content of the primary dopaminergic neurons. All ELISA operations followed the official instructions.

Western Blotting

The cell samples or rat striatum tissues were homogenized in a lysis buffer with protein inhibitor and PMSF (1 mM). The lysates were centrifuged at 1000 rpm for 5 min, and the supernatant was collected. An amount of 20 μg protein samples were separated using 10% SDS-PAGE gel, followed by being transferred onto PVDF membranes. The blots were first blocked in TBST-milk and then incubated with the primary antibody overnight at 4°C. Next, the secondary antibodies conjugated with HRP (1: 1000) were used for 2 h of incubation. The expression of β-actin was used as an endogenous reference. The Tanon ECL Kit was used for chemiluminescence, and the Tanon 5200 chemiluminescence imager was used for image capture.

The primary antibodies applied in this study were as follow: Rabbit monoclonal [EPR19695] to Dopamine Transporter (Abcam, UK, ab184451), Rabbit monoclonal [EPR23457-15] to STXA (Abcam, UK, ab272736), Anti-beta-actin rabbit polyclonal antibody (Abcam, UK, ab8227). Goat anti-rabbit HRP (Abcam, UK, ab6721) was used as the secondary antibody.

CO-IP

For each protein sample, 500 μg protein was collected, and the STX1A antibody (1:30) or DAT antibody (1:30) was added. The rabbit IgG was used as a negative control. Each tube of mixture was slightly shacked overnight at 4°C. The remaining total protein was directly used in Western Blot as an Input control. Next, 40 μl of protein A/G agarose beads was added, and the mixture was shacked at 4°C for 4 h to couple the antibody to the agarose beads. Then, the suspension was centrifuged at 4°C for 3 min (1000 g), the supernatant was carefully aspirated off, and the precipitate was collected for analysis. Subsequently, 500 μl of RIPA lysate was added to the precipitate, and the system was homogenized. The lysates were centrifuged at 4°C for 3 min (1000 g), the supernatant was collected, and this step was repeated 3 times. The collected proteins were further added 5×SDS loading buffer and boiled for 5 min. Next, routine Western blotting analysis was performed.

Adenovirus

Three pairs of Adenovirus were produced by Shanghai Genechem Co., Ltd., including the STX1A-overexpression adenovirus and the corresponding negative control virus (namely, STX1A vs NC), STX1A interfering shRNA adenovirus and corresponding scramble RNA control virus (namely, sh-STX1A vs sh-Control), DAT overexpression adenovirus and corresponding negative control virus (namely, DAT vs NC).

Intra-striatal virus injection

Each rat was fixed on a stereotaxic instrument, a micro-injector was used to inject the Adenovirus into the bilateral striatum (5 μl each side, the virus amount for each rat was 10^10 units). The injection site was as follow: AP + 1.0 mm, ML ± 2.5 mm, DV -3.8 mm. TD modeling was performed on the 3rd day after injection. After the behavioral observation, animals were sacrificed, and brain tissue samples were collected. Brains were fixed in 4% paraformaldehyde, and the reporter gene (GFP) staining was conducted to confirm the accurate injection of the virus.

Immunofluorescence

The rats were transcardially perfused with 0.01M PBS followed by 50 ml of 4% paraformaldehyde in 0.1 M PBS (pH 7.4). The brain tissues were quickly separated and post-fixed in 4% paraformaldehyde overnight, then the brain was dehydrated in 20% sucrose (0.1 M PBS) for 24 h at 4°C and further dehydrated in 30% sucrose (0.1 M PBS) for 24 h at 4°C. The sections were cut into 15 μm sections on a cryostat. The sections were rinsed in 0.01 M PBS and blocked for 2 h with donkey serum (in 0.3% Tween-20 and 0.01 M PBS) and then incubated with rabbit anti STX1A antibody and mouse anti TH antibody (to label dopaminergic neurons) at 4°C overnight (1:500, Proteintech). Subsequently, sections were washed 3 times in 0.01 M PBS for 5 min and incubated with donkey anti-rabbit IgG conjugated with FITC (1:200) and donkey anti-mouse IgG conjugated with CY3 (1:200). Next, sections were incubated with DAPI for nucleus staining for 15 min and washed 3 times for 5 min each. Finally, sections were cover slipped, and images were captured under a fluorescence microscope.

Stereotyped behavior test

The stereotyped behavior test was conducted at 9:00- 11:00. The light condition during the observation period was consistent throughout the experiment. Each rat was placed in the observation box (diameter = 40 cm, height = 30 cm), and the 5-min recording for bite time, head shaking time, and rotation time were conducted by two observers. Finally, the stereotypy score was assessed using the following criterion: No stereotypy or normal activity (score = 0), Discontinuous circling behavior or occasional head twitching (score = 1), Occasionally vertical dyskinetic head and neck movements, occasional sniffing, licking, and biting (score = 2), Continuous circling behavior, increased body raising, increased sniffing, repetitive grooming (such as paw-to-mouth movements) (score = 3), Increased lateral and vertical dyskinetic head and neck movements (score =4).

Statistical analysis

Results were expressed as means ± standard error. For two-group comparison, Student's t-test was used after the normal distribution test. A P value < 0.05 was considered as statistically significant.

Results

TD rats have reduced striatal STX1A expression

First, the stereotypic behavior induced by IDPN was confirmed in our previous study. The TD model rats had more counts in biting, head twitching, shaking claws, continuous rotation, and the stereotypic behavior score. This validated protocol was also reported by similar studies [23, 27, 28]. In this study, we first focused on the striatal STX1A expression of the TD model. The striatal STX1A expression in the protein and mRNA levels (as well as STX1A expression in striatal dopaminergic neurons) were shown in Figure 1. In the immunofluorescence staining of striatal dopaminergic neurons, the TD group showed significantly decreased STXA1 positive and STX1A/TH double positive fluorescence (Figure 1A-C, $P < 0.01$). Consistently, the total STX1A protein (assessed by Western blot, Figure 1D-1E) and STX1A mRNA (assessed by real-time qPCR, Figure 1F) levels were decreased in the TD model rats ($P < 0.01$ and 0.05, respectively). Additionally, there were no changes in the expression of some genes closely related with STX1A, such as SNAP25, SYN, and gephyrin (data not shown). Together, STX1A expression is reduced in the IDPN induced TD individuals.

Changes of in-vitro dopamine distribution and release under the overexpression of STX1A in primary dopaminergic neurons

In vitro, the rat primary dopaminergic neurons were successfully isolated and purified. The cell morphology under the microscope is shown in Figure 2A. The cell body was round or polygonal, and the cells had obvious axons and dendrites. The dendrites ranged from thick to thin, with branches. After cell immunofluorescence identification, all purified cells expressed TH (Figure 2B). After transfection with the rat-STX1A-overexpression adenovirus (the transfection efficiencies of the two groups were similar, Figure 2C), real-time qPCR analysis confirmed that the expression of STX1A mRNA was increased to about 5 times (Figure 2D, $P < 0.01$). Next, we probed the DA levels in the supernatant and the cell lysate. The DA content in the supernatant was significantly lower in the STX1A (overexpressed) group than that the negative control (NC) group (Figure 2E, $P < 0.01$). Meanwhile, the intracellular DA content (assessed based on the cell lysate samples) was significantly higher in the STX1A group (Figure 2F, $P < 0.05$). These results suggested that STX1A may promote the reabsorption of dopamine, and that the down-regulation of STX1A in the TD model may lead to impaired DA reuptake, which further causes a high level of extracellular DA that triggers the TD-like behaviors.

Overexpression of STX1A in vivo partially counteracts the IDPN-induced TD-like behaviors

Furthermore, adenovirus was injected into the rat striatum to overexpress rat STX1A in vivo (Figure 3A), and then the TD model was induced by IDPN 2 days after adenovirus injection. The elevated STX1A expression was verified by real-time qPCR (Figure 3B, $P < 0.01$), Western blot (Figure 3C, $P < 0.05$) and immunofluorescence (Figure 3D-E, $P < 0.01$). Behavioral tests showed that the STX1A group had reduced bite time (Figure 3F, $P < 0.05$) and head shaking time (Figure 3G, $P < 0.01$). There were no significant changes in other TD-like behaviors (such as continuous rotation behavior and stereotypy scores). This result is consistent with the above findings and implies that a sufficient STX1A expression has the effect of resisting the development of TD.

In-vivo knockdown of STX1A aggravates TD-like behaviors

Conversely, adenovirus was injected into the striatum to knock down STX1A expression in vivo, and then the TD model was induced after two days of recovery. The decreased STX1A expression was verified by real-time qPCR (Figure 4A, $P < 0.01$), Western blot (Figure 4B, $P < 0.01$) and immunofluorescence (Figure 4C-D, $P < 0.01$). The sh-STX1A group had significantly aggravated TD-like behaviors versus sh-Control, including the significant increases in bite time (Figure 4E), head shaking time (Figure 4F), continuous rotation behavior (Figure 4G), and the stereotypy score (Figure 4H). These results revealed that when the expression level of STX1A is insufficient, the IDPN-induced TD-like behavior can be further aggravated. And it supports the above conclusion that the striatal STX1A may play an anti-TD role.

Overexpression of DAT in vivo alleviates TD-like behavior and increases STX1A expression

Given that overexpression of STX1A can reduce the DA content of the supernatant and increase the intracellular DA content (Figure 2), we hypothesized that STX1A may be associated with DA reuptake and interact with DAT. Similarly, we overexpressed DAT in the striatum using the rat-DAT-overexpression adenovirus. The expression of DAT was verified by immunofluorescence (Figure 5A & 5B). Next, we observed the expression of STX1A and TD-like behavior. Interestingly, overexpression of DAT in the striatum resulted in increased levels of STX1A mRNA (Figure 5C, $P < 0.05$) and protein (Figure 5D, $P < 0.01$). This result suggests that there may indeed be a protein-protein interaction between STX1A and DAT. Moreover, in the behavioral test, the DAT (overexpressed) group exhibited reduced bite time (Figure 5E, $P < 0.05$), a slightly (without significant difference) decrease in head shaking time (Figure 5F), decreased continuous rotation time (Figure 5G, $P < 0.05$), and highly significantly improved stereotypy score (Figure 5H, $P < 0.01$). Collectively, DAT can affect the expression of STX1A, and a high expression of DAT has an anti-TD effect.

DAT and STX1A binding analysis

In order to clarify the interaction between DAT and STX1A, we extracted the total protein of purified striatal dopaminergic neurons and conducted the CO-IP analysis. Compared to the IgG control, the blots of DAT and STX1A showed significant binding to each other (Figure 6A). This result confirmed that the protein-protein interaction between DAT and STX1A in striatal dopaminergic neurons is a mechanism of the expression-modulation influence and the effects on DA reuptake.

Discussion

In this study, we used a rat TD model induced by IDPN to observe the role of striatal STX1A in TD-like behaviors. Our main findings are: (1) TD rats have reduced striatal STX1A expression; (2) overexpression STX1A promotes dopamine reuptake; (3) Overexpression of STX1A in vivo partially counteracts the IDPN-induced TD-like behaviors, while In-vivo knockdown of STX1A aggravates TD-like behaviors; (4) Overexpression of DAT in vivo alleviates TD-like behavior and increases STX1A expression; (5) DAT and STX1A have a protein interaction. From the above results, the pathogenesis of TD and the mechanism of influence of STX1A on TD-like behavior are proposed as follow (summarized in Figure 6B). Under the condition of IDPN-induced TD, the expression of STX1A in the striatum is impaired, which in turn affects

the reuptake of DA by DAT; to be more specific, combination of STX1A and DAT is crucial in DA reuptake, impaired STX1A expression cause an accumulation of DA in the synaptic cleft which triggers an abnormal DA signal activation in TD development; and the exorbitant DA signal drives the pathological stereotyped behavior. However, this hypothesis urgently needs experimental confirmation.

STX1A encodes a component of the presynaptic SNARE complex, and it is closely associated with presynaptic vesicle release. It participates in serotonin transporter regulatory, glutamate transport and γ-aminobutyric acid (GABA) transport [29, 30]. STX1A might influence the serotonergic system during neurodevelopment [30]. It is known that ablation of STX1A may cause disruption of 5-HT-ergic transmission and induce abnormal behavior. Moreover, the JNK2/STX1 interaction is involved in presynaptic NMDA-evoked glutamate release [31]. In the parkinsonian animal model induced by amphetamine, the expression of STX1A decreased in the nucleus accumbens (NAc) while increased in the shell region [32]. Similar to Parkinson's disease, the relationship between STX1A and central nervous system diseases is increasingly noticed [30, 33]. Moreover, known disease associated with STX1A include Williams Syndrome, autism, Asperger syndrome, children attention-deficit/hyperactivity disorder, and cryptogenic epilepsy [30, 33–37].

To date, the functional relationship between STX1A and dopaminergic neurons is not fully clear, but the interaction of STX1A to DAT has been noticed in other fields. Referential findings are as follow. The DAT reuptakes dopamine into presynaptic neurons through sodium-dependent calcium channels to regulate the intensity and duration of dopamine signal activation, and STX1A is believed to be involved in this process [38]. DAT/STX1A interaction can regulate the activity of transport channels and dopaminergic synaptic transmission [39]. Another study showed that combination of STX1A and DAT can promote the release of dopamine, and amphetamine can facilitate their combination, which is mediated by CAMKII [40]. Additionally, a study in 2010 used botulinum neurotoxin C to treat rat striatum tissue, it revealed the binding effect of STX1A and DAT and that STX1A can modulate DA by influencing the re-uptake role of DAT [41]. However, in recent years, limited studies have been published about the relationship between DAT and STX1A. Together, these literatures indicate that STX1A may affect DA reuptake through DAT, and it may regulate the balance of the dopaminergic system. However, to our best knowledge, no studies have observed the change of STX1A in the striatum tissue of TD individuals.

Given the clear function dopamine release and striatal pathways in TS/TD [42–45], the role of DAT in TS/TD or similar stereotypic behaviors is also a conceivable concern. So far, it has been widely accepted. For example, DAT is involved in methamphetamine-induced behavioral sensitization in tree shrews [46]. DAT KO mice exhibited a highly stereotyped consummatory behavior; increased dopamine in DAT KO mice not only increased perseveration of bouts and individual lick duration, but also increased the behavioral variability in response to the extinction contingency and the rate of extinction [47]. Moreover, DAT inhibition is necessary for cocaine-induced stereotypy in mice [48]. Notably, an evaluation of the serotonin system and perseverative, compulsive, stereotypical, and hyperactive behaviors was found in the DAT-knockout mice [49]. However, above findings have not shown the role of striatal DAT or DA. Recently, a Chinese herbal prescription was reported to upregulates the striatal DAT and attenuates

stereotyped behavior of Tourette syndrome in rats [50]. In addition, another traditional Chinese medicine was noticed to exert the protective and restorative effects against methamphetamine-induced dopaminergic neurotoxicity: it attenuates the stereotyped responses and restores DAT expression to the normal level [51]. Together, it is a reliable belief that DAT inhibition, as well as the resulting DA accumulation, is a key mechanism underlying TD development.

Still, the present study has some limitations. First, due to the time limitation, we have not performed the test about whether direct interference of STX1A (versus the normal control) may cause TD-like behaviors. Therefore, it is still unclear the necessary role of STX1A in the pathological process of TD. Besides, the specific binding manner of the two proteins is not clear in this study, and further exploration is still needed.

Conclusion

Striatal STX1A expression is decreased in TD development, and STX1A plays an anti-TD role possibly through interaction with DAT, which maintains the DA reuptake. The exorbitant DA signal caused by STX1A inhibition drives the pathological stereotyped behavior.

Declarations

Data Availability

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Author contributions

Xiumei Liu and Xueming Wang conceived and designed the study. Xiumei Liu andXueming Wang performed the experiments, analyzed the data, and wrote the paper, Aihua Cao and Xiaoling Zhang performed the experiments, analyzed the data. All authors declared that they read and approve manuscript final version.

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Compliance with ethical standards

Conflict of interest

All the authors declare that they have no conflict of interest or financial ties to disclose.

Consent for publication

All authors gave consent for this manuscript to be published.

Ethical approval

This study was approved by the Ethical Committee (UERC) of Fujian Provincial Children's Hospital.

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Figures

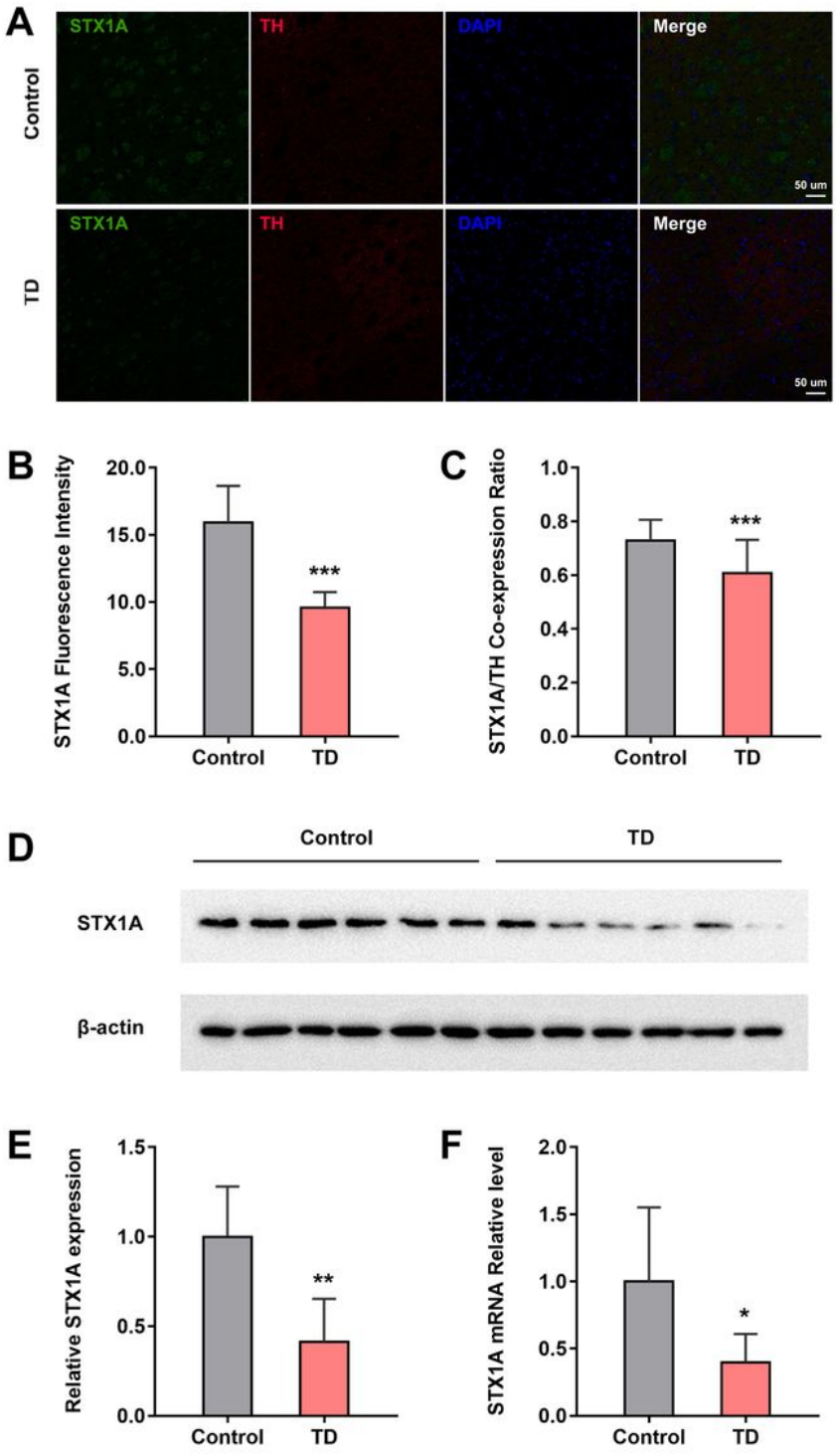

Figure 1

STX1A is decreased in the striatum of Tic disorder (TD) rats. (A) The immunofluorescence staining of striatal dopaminergic neurons. (B) The TD group has decreased STXA1 fluorescence. (C) The TD group has decreased STX1A/TH double positive fluorescence. (Figure 1 A-C, $P < 0.01$). (D) The total STX1A protein in the striatum (assessed by Western blot) is decreased in the TD group. (E) The statistical

analysis of STX1A protein in the striatum. (F) The STX1A mRNA level (assessed by real-time qPCR) is decreased in the TD group. * P < 0.05, ** P < 0.01, *** P < 0.001.

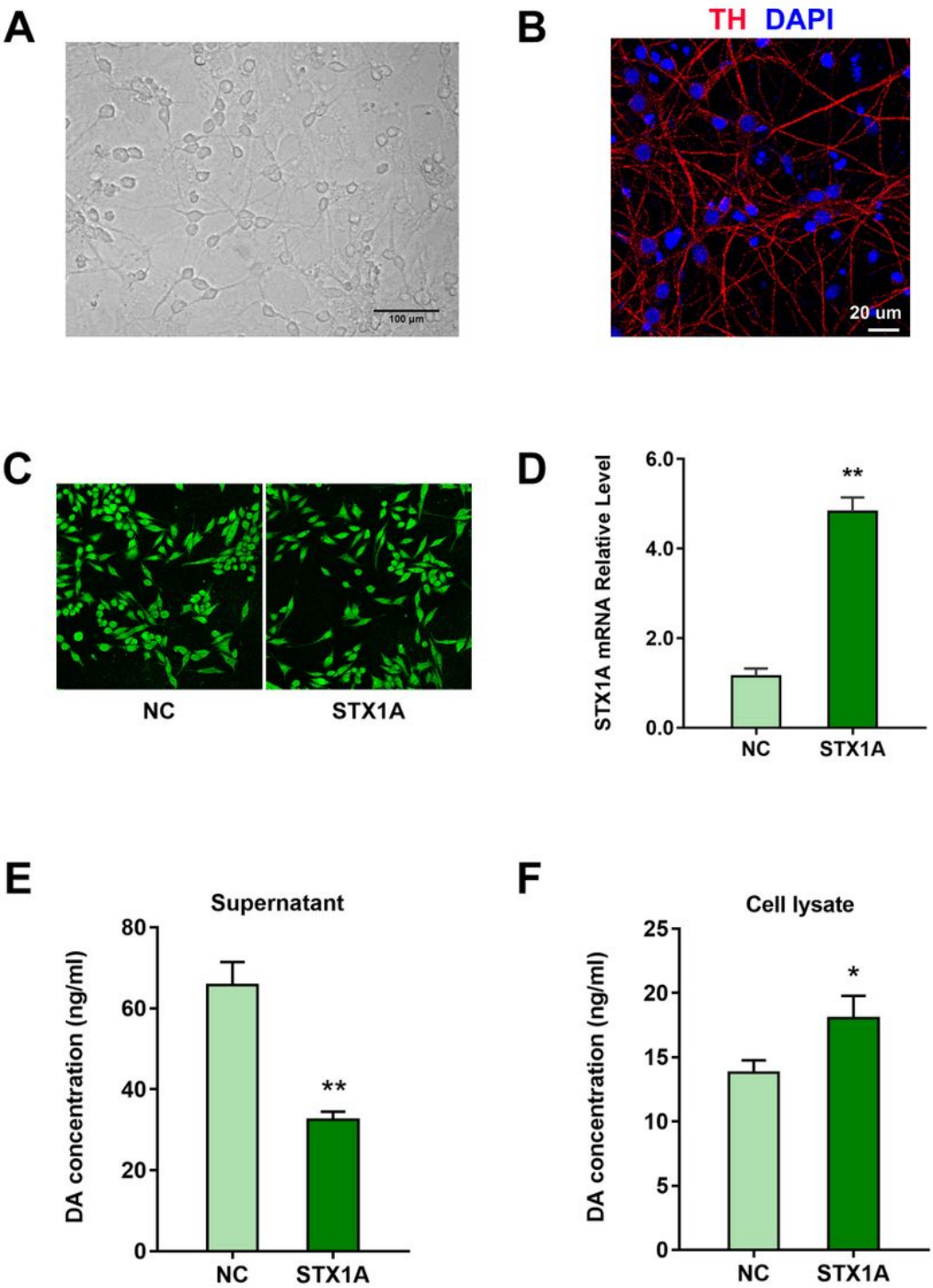

Figure 2

Changes of in-vitro dopamine (DA) distribution and release under the overexpression of STX1A in primary dopaminergic neurons. (A) In vitro, the rat primary dopaminergic neurons were successfully isolated and

purified. The cell morphology under the microscope. (B) The cell immunofluorescence identification shows all purified cells expressed TH. (C) Identification of the transfection efficiencies of the rat-STX1A-overexpression adenovirus and the control adenovirus (both at least 90%). (D) Real-time qPCR analysis of the expression of STX1A mRNA. The STX1A (overexpressed) group has 5 times of the negative control (NC). (E) The DA content in the supernatant is significantly lower in the STX1A group than that the NC group. (F) Meanwhile, the intracellular DA content (assessed based on the cell lysate samples) is significantly higher in the STX1A group. * P < 0.05, ** P < 0.01.

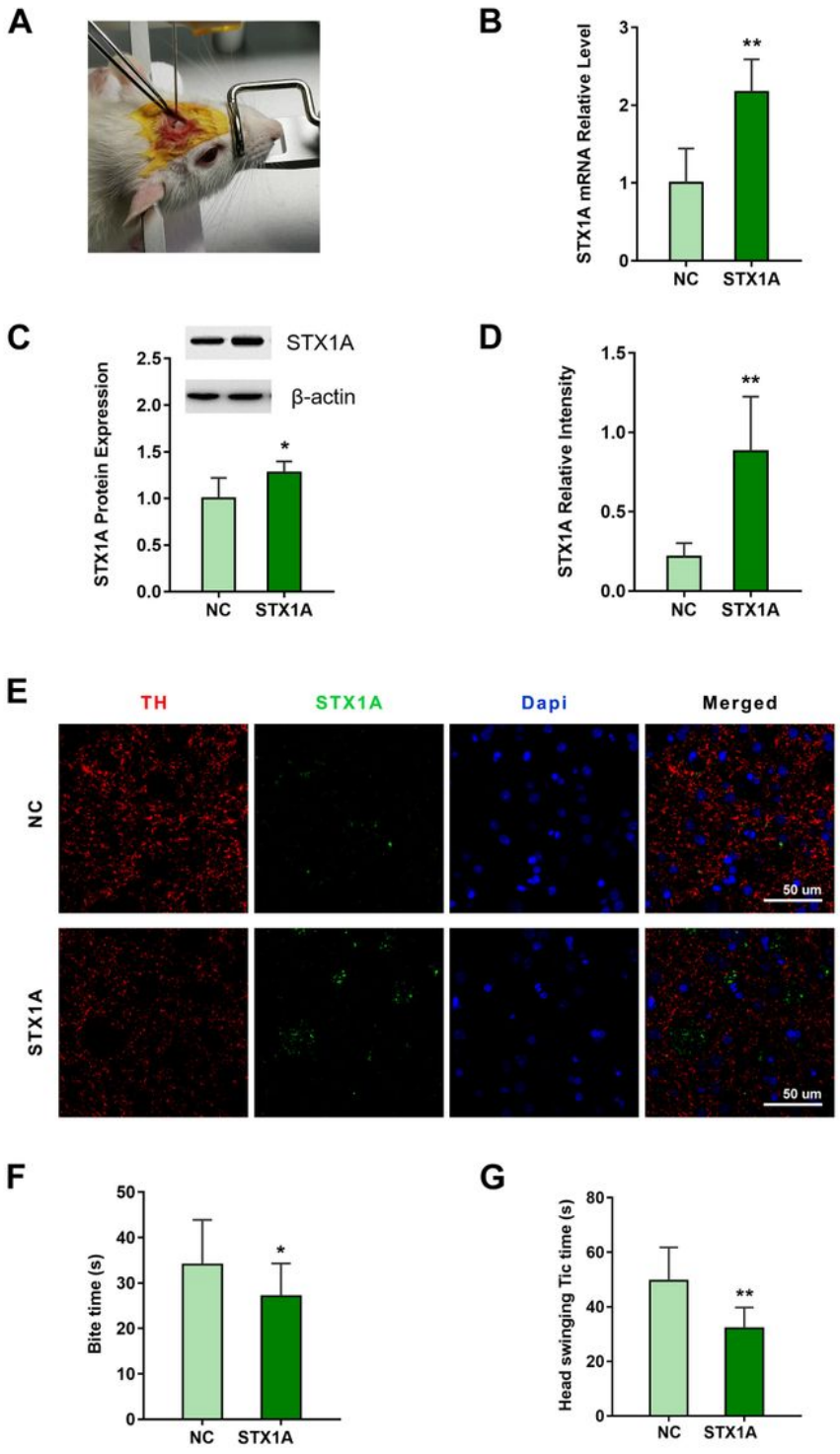

Figure 3

Overexpression of STX1A in vivo partially counteracts the IDPN-induced TD-like behaviors. (A) Adenovirus is injected into the rat striatum to overexpress rat STX1A in vivo (AP + 1.0 mm, ML ± 2.5 mm, DV -3.8 mm). (B) The elevated STX1A mRNA expression verified by real-time qPCR., (C) The elevated STX1A protein expression verified by Western blot. (D-E) The elevated STX1A mRNA expression verified by immunofluorescence. (F) The behavioral test after TD modeling: bite time. (G) The behavioral test after TD modeling: head shaking time. * $P < 0.05$, ** $P < 0.01$.

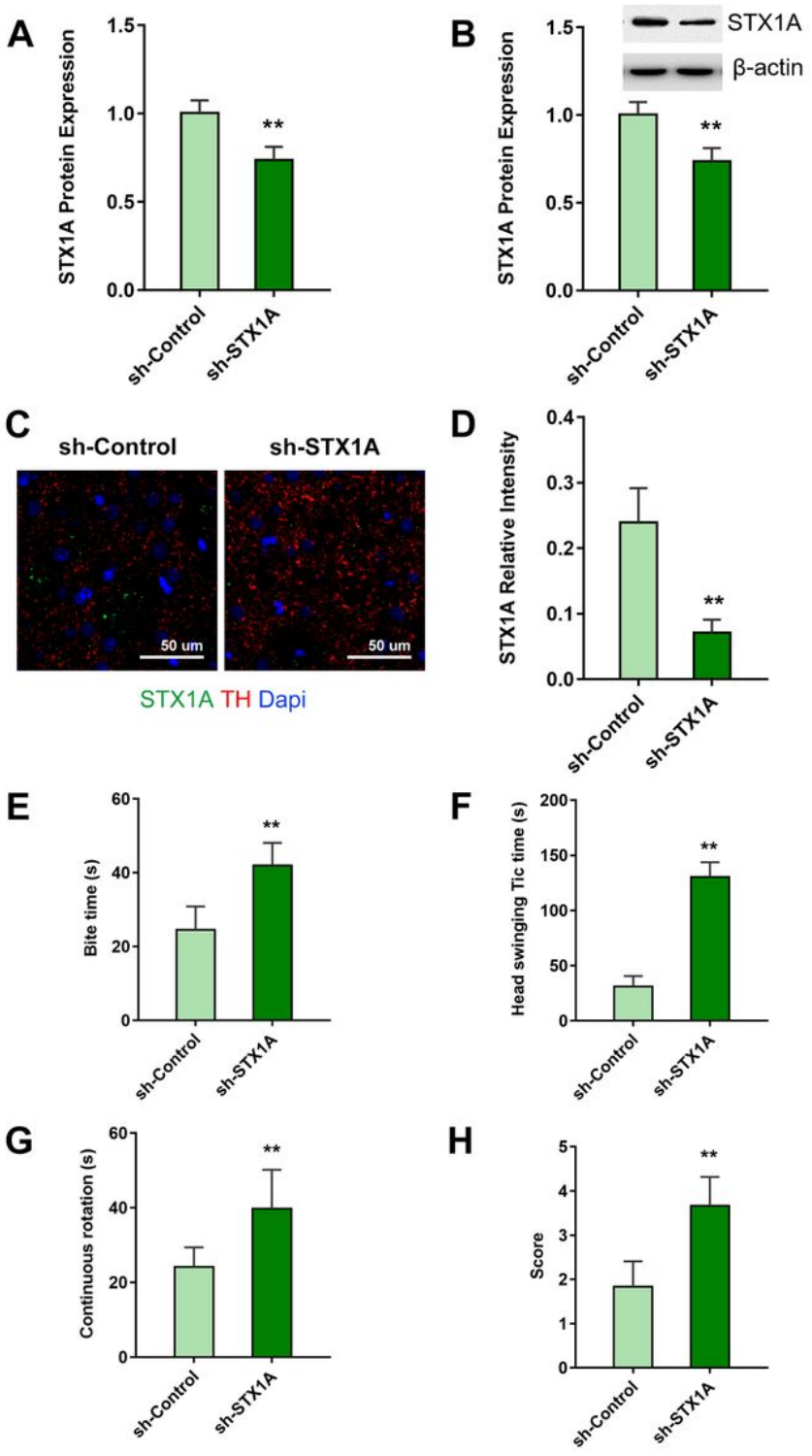

Figure 4

In-vivo knockdown of STX1A aggravates TD-like behaviors. Adenovirus loading STX1A shRNA was injected into the striatum to knock down STX1A expression in vivo, and then the TD model was induced after two days of recovery. (A) The decreased STX1A mRNA expression verified by real-time qPCR (B) The decreased STX1A protein expression verified by Western blot. (C-D) The immunofluorescence staining shows the sh-STX1A group has decreased STX1A expression. In the behavioral test, the sh-STX1A group

has significant increases in (E) bite time, (F) head shaking time, (G) continuous rotation behavior, and (H) the stereotypy score. * P < 0.05, ** P < 0.01.

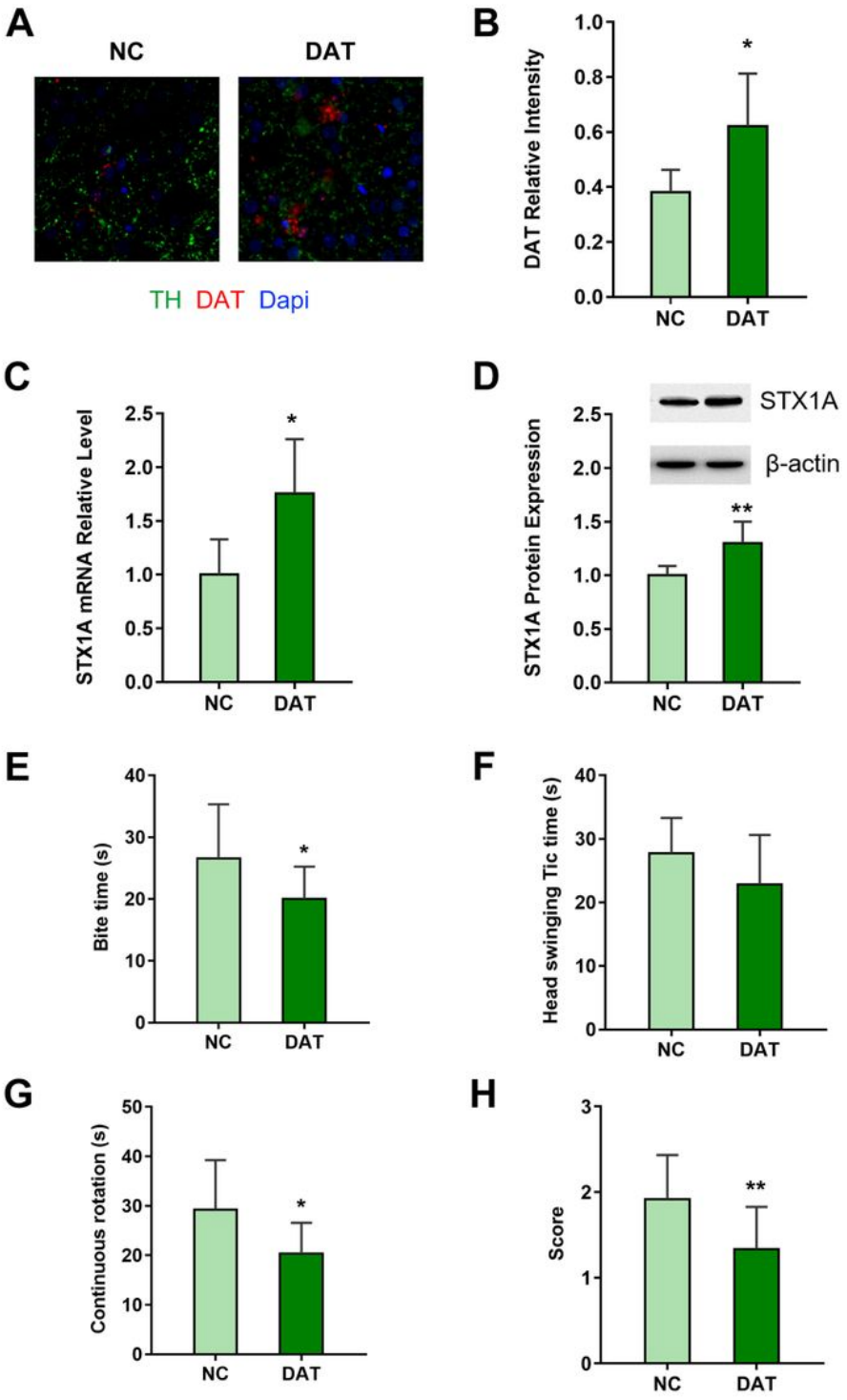

Figure 5

Overexpression of DAT in vivo alleviates TD-like behavior and increases STX1A expression. Similarly, we overexpressed DAT in the striatum using the rat-DAT-overexpression adenovirus. (A-B) The elevated

expression of DAT verified by immunofluorescence. (C) Overexpression of DAT in the striatum results in increased levels of STX1A mRNA. (D) Overexpression of DAT causes increased expression of STX1A protein. In the behavioral test, the DAT (overexpressed) group exhibits: (E) reduced bite time, (F) a slightly decrease in head shaking time (P > 0.05), (G) decreased continuous rotation time, and (H) highly significantly improved stereotypyscore. * P < 0.05, ** P < 0.01.

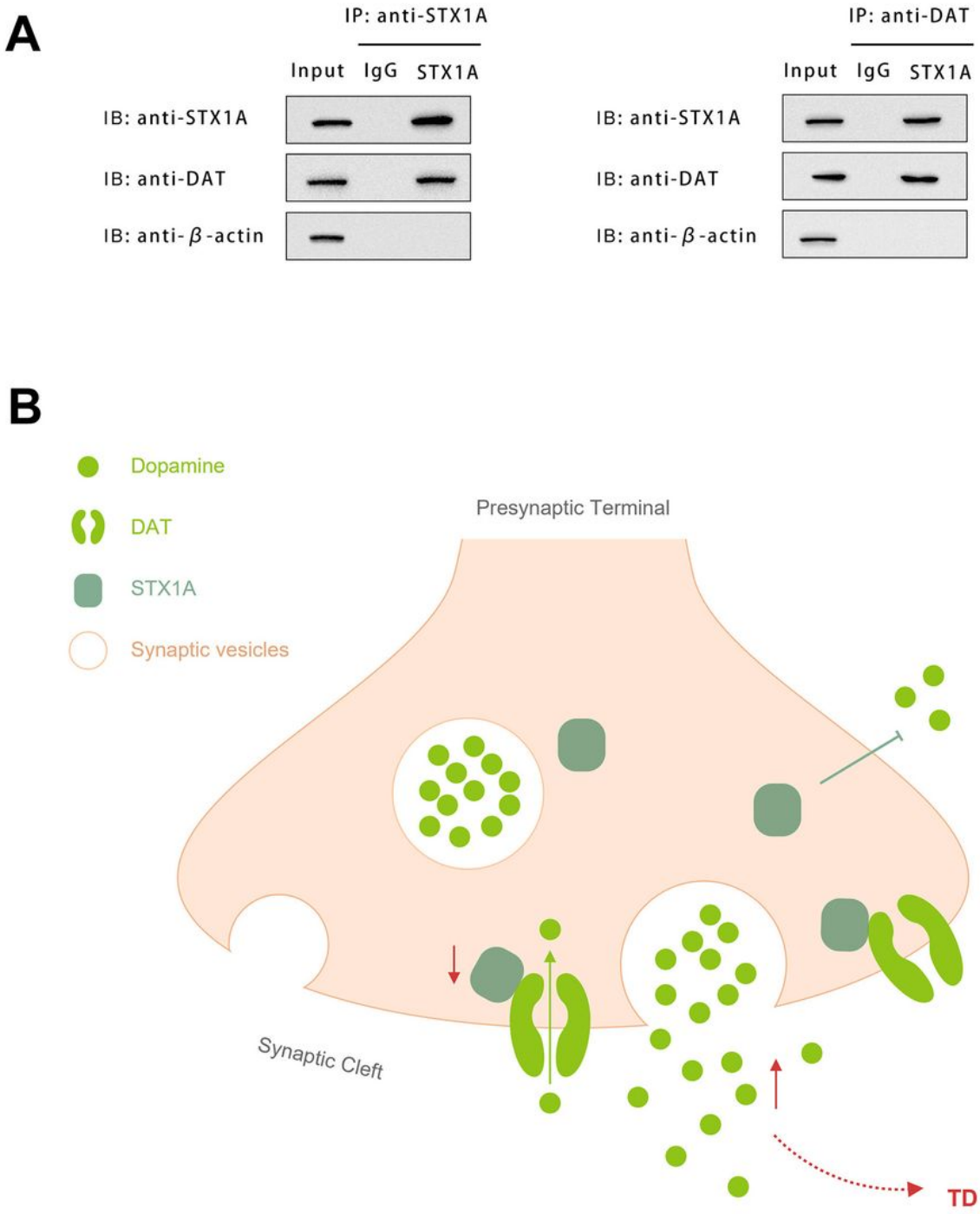

Figure 6

CO-IP analysis of DAT and STX1A and the mechanism summary diagram. (A) Total protein from purified striatal dopaminergic neurons is conducted the CO-IP experiment. Compared to the IgG control, the blots of DAT and STX1A have significant binding to each other. (B) Schematic diagram of the proposed mechanism. Under the condition of IDPN-induced TD, the expression of STX1A in the striatum is impaired, which in turn affects the reuptake of DA by DAT; to be more specific, combination of STX1A and DAT is crucial in DA reuptake, impaired STX1A expression can cause an accumulation of DA in the synaptic cleft which triggers an abnormal DA signal activation in TD development; and the exorbitant DA signal drives the pathological stereotyped behavior.