RESEARCH ARTICLE

Characteristic response of striatal astrocytes to dopamine depletion

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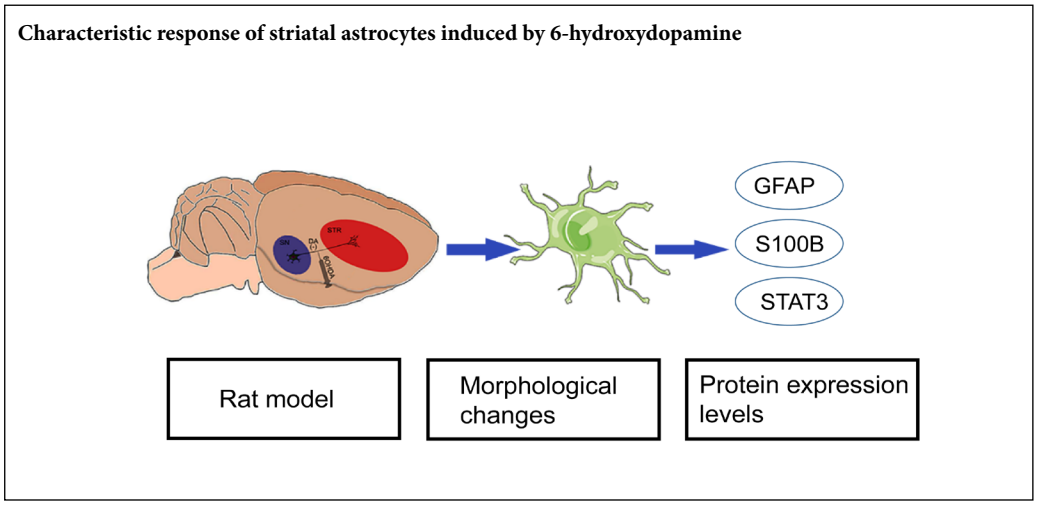
Graphical Abstract



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Abstract

Astrocytes and astrocyte-related proteins play important roles in maintaining normal brain function, and also regulate pathological processes in brain diseases and injury. However, the role of astrocytes in the dopamine-depleted striatum remains unclear. A rat model of Parkinson's disease was therefore established by injecting 10 μL 6-hydroxydopamine (2.5 μg/μL) into the right medial forebrain bundle. Immunohistochemical staining was used to detect the immunoreactivity of glial fibrillary acidic protein (GFAP), calcium-binding protein B (S100B), and signal transducer and activator of transcription 3 (STAT3) in the striatum, and to investigate the co-expression of GFAP with S100B and STAT3. Western blot assay was used to measure the protein expression of GFAP, S100B, and STAT3 in the striatum. Results demonstrated that striatal GFAP-immunoreactive cells had an astrocytic appearance under normal conditions, but that dopamine depletion induced a reactive phenotype with obvious morphological changes. The normal striatum also contained S100B and STAT3 expression. S100B-immunoreactive cells were uniform in the striatum, with round bodies and sparse, thin processes. STAT3-immunoreactive cells presented round cell bodies with sparse processes, or were darkly stained with a large cell body. Dopamine deprivation induced by 6-hydroxydopamine significantly enhanced the immunohistochemical positive reaction of S100B and STAT3. Normal striatal astrocytes expressed both S100B and STAT3. Striatal dopamine deprivation increased the number of GFAP/S100B and GFAP/STAT3 double-labeled cells, and increased the protein levels of GFAP, S100B, and STAT3. The present results suggest that morphological changes in astrocytes and changes in expression levels of astrocyte-related proteins are involved in the pathological process of striatal dopamine depletion. The study was approved by Animal Care and Use Committee of Sun Yat-sen University, China (Zhongshan Medical Ethics 2014 No. 23) on September 22, 2014.

Key Words: *6-hydroxydopamine; astrocyte; dopamine depletion; dopaminergic neurons; Parkinson's disease; S100B; STAT3; striatum*

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Introduction

Increasing evidence has revealed that the degeneration of midbrain dopamine (DA) neurons that characterizes Parkinson's disease (PD) begins in the striatal synaptic terminals of DA neurons and progresses retrograde to cell soma in the pars compacta of the substantia nigra ("dying-back degeneration") (Kordower et al., 2013; Grosch et al., 2016; Morales et al., 2016; Tagliaferro and Burke, 2016; Gao et al., 2019; Goldstein and Sharabi, 2019). The degeneration of DA neurons induces DA depletion in the striatum, which results in an imbalance of inhibitory and excitatory inputs in striatal medium spiny neurons, ultimately leading to the dysfunction and degeneration of striatal neurons (Deutch et al., 2007; Shen et al., 2008). Increasing evidence suggests that striatal astrocytes are involved in this degeneration (Privat, 2003; Wang and Bordey, 2008; Lee et al., 2010). Astrocytes play an important role in synaptic and neuronal functions, and provide metabolic, structural, and trophic support (O'Neill and Harkin, 2018). Moreover, astrocytes maintain homeostatic conditions for synapses and neurons by regulating neurotransmitters and extracellular ions (Covelo and Araque, 2016). A growing body of literature has investigated whether astrocytes are associated with the regulation of neuronal and synaptic functions, influencing and controlling neuronal activity and behavior by responding to neurotransmitter release (from synaptic terminals) and glial-derived factors (Araque et al., 2001; Nedergaard et al., 2003; Volterra and Meldolesi, 2005; Parpura and Zorec, 2010; Araque et al., 2014). The present study aimed to characterize the response of striatal astrocytes to DA depletion in a rat model of PD.

The role of striatal astrocytes in the DA-depleted striatum is not well known. Some neurodegenerative diseases may be associated with over reactive astrocytes (Teismann et al., 2003; Halliday and Stevens, 2011; Pekny and Pekna, 2014; Shi and Chen, 2017; Kumar and Kumar, 2018). Neurodegenerative disease pathologies are often activated by glial cells in the brain, and this is known as "reactive gliosis" (Pekny and Nilsson, 2005). Reactive gliosis may produce neurotrophic factors and promote mitochondrial damage and oxidative stress. Previous studies have reported that glial fibrillary astrocytic protein (GFAP) immunoreactivity increased significantly in the substantia nigra and striatum in the brain of both PD patients and the 6-hydroxydopamine (6-OHDA)-induced PD rat model (Dong and Benveniste, 2001; Mythri et al., 2011). A large number of studies have shown that astrocytes express abnormal inflammatory factors that participate in the pathology of neuronal damage (Yan et al., 2014). Although the degeneration of the dopaminergic system is accompanied by activation of the surrounding astrocytes (Walsh et al., 2011), the indirect effects of DA depletion on striatal astrocytes are unclear. We injected 6-OHDA into the medial forebrain bundle, which has been reported to degenerate the striatal dopaminergic innervation, but which prevents direct damage to the striatum (Ma et al., 2014). This PD model was used here to characterize the striatal astrocytic response to the indirect effects of DA depletion.

Reactive gliosis involves the overexpression of glial-derived factors. Calcium-binding protein B (S100B) is strongly associated with the pathological process of PD (Pekny and Pekna, 2014). S100B is secreted by astrocytes in the central nervous system and plays a regulatory role in the cytoskeleton and cell cycle (Donato et al., 2009). S100B may also participate in 6-OHDA-induced and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced dopaminergic neurodegeneration (Muramatsu et al., 2003; Chadi and Gomide, 2004; Himeda et al., 2006). However, the characteristic response of S100B-expressing astrocytes to striatal DA depletion is not well known. Convincing evidence indicates that the S100B protein accumulates at the receptor for advanced glycation end-products (RAGE), resulting in the phosphorylation of mitogen-activated protein kinase and the activation of nuclear factor-kappa B (Schmidt et al., 2001; Cirillo et al., 2011). However, the characteristic response of astrocytes and signal transducer and activator of transcription (STAT) to striatum dopaminergic desensitization is unknown. The STAT protein family mediates apoptosis, cellular immunity, proliferation, and differentiation (Vinkemeier et al., 1998). There is increasing evidence that STAT3 also plays an important role in astrocyte function. The STAT3-mediated activation of astrocytes has been reported in rodent models of PD, cerebral ischemia, epilepsy, and spinal cord injury (Choi et al., 2003a, 2003b; Sriram et al., 2004; Su et al., 2010). In addition, a previous study indicated that astrocytic cells activate the neurotoxicity of the Janus kinase (JAK)–STAT3 pathway (Hashioka et al., 2015). Reactive gliosis is a heterogeneous phenomenon that induces functional and structural changes in the brain, and its degree and effects manifest in a cell-context-dependent manner. A further aim of this study was thus to test whether DA depletion affects the expression of GFAP, S100b, and STAT3 proteins in the striatum.

Materials and Methods

Experimental animals

Eight-week-old specific-pathogen-free male Sprague-Dawley rats weighing 200–250 g were used for this study. All rats were obtained from the Center for Experimental Animals of Sun Yat-sen University, China (license No. SYXK (Yue) 2015-0107). All rat care and experimental procedures were approved by the Animal Care and Use Committee of Sun Yat-sen University, China (Zhongshan Medical Ethics 2014 No. 23) on September 22, 2014. Animal welfare and experimental procedures were conducted in strict accordance with the Guide for the Care and Use of Laboratory Animals (Guide for the Ethical Review of Animal Welfare of China, 2018). The rats were individually housed in an air-conditioned room (22 ± 0.5°C) under a 12-hour light–dark cycle with water and food available *ad libitum*. All surgeries were performed under anesthesia using sodium pentobarbital. All efforts were made to minimize animal suffering and to reduce the number of animals used.

The Sprague-Dawley rats (n = 24) were randomly assigned to the normal control group (n = 8), the 6-OHDA group (n = 8), of the vehicle control group (n = 8). In each group, four rats were used in immunohistochemical experiments and four rats were used in western blot assays.

Treatment of animals

6-OHDA, a neurotransmitter analog, was used to induce nigrostriatal DA depletion (Jia et al., 2014). Rats were deeply anesthetized with sodium pentobarbital (50 mg/kg, intraperitoneally) and fixed on a Kopf stereotaxic frame (Stoelting, Kiel, WI, USA). Burr holes were made in the skull overlaying the right medial forebrain bundle. The rats in the 6-OHDA group were injected with 10 μL 6-OHDA (2.5 μg/μL; Sigma, St. Louis, MO, USA; catalog No. H116; dissolved in 0.9% saline) in the right medial forebrain bundle (anteroposterior: –3.6, mediolateral: –0.19, dorsoventral: –8.2 from bregma) using a syringe (Hamilton, Reno, NV, USA). Rats in the normal control group did not receive any surgery or treatment, while rats in the vehicle control group received 10 μL vehicle (0.9% saline) in the right medial forebrain bundle.

Apomorphine-induced rotation (Tocris, Bristol, UK) was measured 3 weeks after the 6-OHDA injection to the medial forebrain bundle. During the 3 weeks following the induction of the 6-OHDA lesion, rats (weekly) were subcutaneously injected with apomorphine (catalogue No. 2073/50, Tocris) at a dose of 0.25 mg/kg. The number of 360° contralateral rotations was counted within 30 minutes. Only rats that completed more than 210 rotations were included for further experiments. The methods used in the present study were previously described in detail (Jia et al., 2014). All rats were sacrificed 28 days after surgery, and immunohistochemical staining of tyrosine hydroxylase was performed to ensure the success of the model. Only rat models with very few tyrosine hydroxylase-immunoreactive fibers in the right striatum were used in the subsequent experiments. The extent of tyrosine hydroxylase-immunoreactive fibers after 6-OHDA lesion development was demonstrated in our previous study (Ma et al., 2014).

Immunohistochemistry

Rats were perfused with 0.9% sodium chloride solution (400 mL) followed by fixative (400 mL, 4% paraformaldehyde, pH 7.4). Brain tissue was immediately removed and immersed in 4% paraformaldehyde overnight at 4°C. Brain that contained striatal tissue was cut into 30 μm sections using a vibratome. The sections were pretreated with 0.3% H_2O_2 at room temperature for 30 minutes. To perform conventional immunohistochemistry, sections were incubated overnight at 4°C with primary antibody. The primary antibodies used were mouse anti-GFAP (1:5000, Cat# MAB3402; Millipore, Boston, MA, USA), rabbit anti-S100B (1:400, Cat# ab52642; Abcam, Shanghai, China), and rabbit anti-STAT3 (1:200, Cat# 8768S; Cell Signaling Technology, Danvers, MA, USA). After rinsing, sections were incubated for 2 hours at room temperature with secondary antibodies: goat anti-mouse IgG (1:200, Cat# 62-6540; Millipore) was used for GFAP and goat anti-rabbit IgG (1:200, Cat# BA1000; VECTOR labs, Burlingame, CA, USA) was used for S100B and STAT3. Sections were then incubated with Avidin-Biotin Solution (1:100; VECTOR labs; Cat# pk-6100) for 2 hours. After rinsing with 0.1 M phosphate buffer, the sections were immersed in enhanced 3,3′-diaminobenzidine (0.05%, Cat# D5637; Sigma) to visualize the staining. The brain sections were then dehydrated, cleared, and coverslipped.

To perform conventional double-label immunofluorescence, sections were incubated overnight at 4°C with mouse anti-GFAP (1:1000, Cat# MAB3402; Millipore) and either rabbit anti-S100B (1:400, Cat# ab52642; Abcam) or rabbit anti-STAT3 (1:200, Cat#8768S; Cell Signaling Technology). Sections were then incubated with anti-mouse fluorescent IgG (1:200, Cy3, Cat# C2181 MSDS; Sigma) and anti-rabbit fluorescent IgG (1:200, Cy5, Cat# SAB4600398 MSDS; Sigma) at room temperature for 2 hours. Sections containing the striatum were observed using a confocal microscope (Nikon C2, Tokyo, Japan).

The sections from each rat were analyzed appropriately for the labeling type in each group. For rats used for immunohistochemical staining, five brain sections were selected from each rat in each group for the statistical analysis. Five non-overlapping 0.01 mm^2 squares were randomly selected in each measured region to count stained cells. The data collection area was the zone of the dorsolateral striatum, which was described in our previous study (Ma et al., 2014). The dorsolateral striatum receives convergent excitatory afferents from the thalamus and cortex, and forms the origin of the indirect and direct pathways (Kreitzer and Malenka, 2008). The dorsolateral striatum is involved in motor control and procedural memory.

For immunohistochemical data, the average optical density of GFAP-immunoreactive cells and the size of GFAP-immunoreactive soma were measured using an image analysis program (Image J v1.8.0) in five randomly selected areas (0.01 mm^2 each) in the dorsolateral striatum. Astrocytic activation was also quantified in the ipsilateral striatum. The numbers of GFAP-, S100B-, and STAT3-immunoreactive cells were counted in five randomly selected areas (0.01 mm^2 each) in the dorsolateral striatum of each section (n = 5, each rat). The investigators were blinded while analyzing morphological studies.

Western blot assay

Rats (n = 4, each group) were deeply anesthetized with sodium pentobarbital (50 mg/kg, intraperitoneally). Striatal tissue was extracted and lysed for further analysis. The protein sample was separated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (10%) and transferred onto a polyvinylidene difluoride membrane (Roche, Mannheim, Germany). Next, the membranes were blocked with 5% skim milk powder in Tris buffered saline-Tween 20 for 1.5 hours, and then incubated with shaking overnight at 4°C with the following primary antibodies: mouse anti-GFAP (1:8000; Millipore), rabbit anti-S100B (1:1500, Cat# ab52642; Abcam), and rabbit anti-STAT3 (1:1500, Cat# 8768S; Cell Signaling Technology). The membranes were then incubated with goat-anti-rabbit IgG antibodies (1:5000, Cat# AP307P; Millipore) and goat anti-mouse IgG (1:200, Cat# 62-6540, Millipore) for 2 hours at room temperature. Protein bands were visualized using the Bio-Rad system (Bio-Rad Laboratories, Shanghai, China) and quantified using ImageJ soft-

ware (National Institutes of Health, New York, NY, USA).

Statistical analysis

All statistical analyses were performed using SPSS Statistics v22.0 software (IBM, Armonk, New York, NY, USA). Data are expressed as the mean ± SD. Comparisons among groups were examined using one-way analysis of variance and independent-samples *t*-tests. A value of $P < 0.05$ was considered statistically significant.

Results

Statistical analyses revealed no significant differences between the normal control and vehicle control groups. Accordingly, we have presented data and statistical analyses for the control and 6-OHDA groups only.

Morphological changes in striatal GFAP-, S100B- and STAT3-immunoreactive cells following DA depletion

Immunohistochemical results showed that astrocytes in control animals were clearly GFAP-immunoreactive with sparse, thin processes and small cell bodies. However, in the 6-OHDA group, the GFAP-immunoreactive cells displayed a reactive phenotype, with thick processes and enlarged cell bodies (**Figure 1**). Quantitative data for the average optical density of GFAP-immunoreactive entities (including cell bodies and processes) demonstrated that, in the dorsal lateral striatum, the average optical density of GFAP-immunoreactive cells was significantly increased in the 6-OHDA group (13.50 ± 0.54/0.01 mm^2), compared with the control group (11.31 ± 0.47/0.01 mm^2; $P = 0.0087$, $n = 20$. In addition, the number of GFAP-immunoreactive cells was higher in the 6-OHDA group (20 ± 0.86/0.01 mm^2) than in the control group (14 ± 1.1/0.01 mm^2; $P = 0.0009$, $n = 20$). Moreover, GFAP-immunoreactive soma were significantly enlarged in the 6-OHDA group (8.63 ± 0.44 μm), compared with controls (4.74 ± 0.40 μm; $P = 0.0001$, $n = 20$).

The normal striatum displayed S100B and STAT3 immunoreactivity, which was evenly distributed in the striatum in cells with small somas and short, sparse, and thin processes (**Figure 2**). However, some STAT3-immunoreactive cells displayed intense staining in cells with large somas that were morphologically consistent with interneurons (**Figure 2C′**). Statistical analysis revealed that S100B-immunoreactive cells were significantly increased in the 6-OHDA group compared with the control group ($P = 0.0256$, $n = 20$; **Figure 3A**). The number of STAT3-immunoreactive cells was also higher in the 6-OHDA group than in the control group ($P = 0.0018$, $n = 20$; **Figure 3A**).

Effects of striatal DA depletion on S100B- and STAT3-expressing astrocytes

Immunofluorescence double-labeling was used to investigate the coexistence of GFAP–S100B and GFAP–STAT3 in striatal astrocytes, as well as the effect of striatal DA depletion on these proteins. Striatal astrocytes (GFAP immunoreactive cells) expressed S100B and STAT3 proteins in both the control and 6-OHDA groups (**Figure 4**). STAT3-immunoreactive neurons were also visible. Quantitative data showed that the number of GFAP–S100B double-labeled cells was significantly increased in the 6-OHDA group compared with controls ($P = 0.0001$, $n = 20$). Similarly, there were more GFAP–STAT3 double-labeled cells in the 6-OHDA group than in the control group ($P = 0.0096$, $n = 20$; **Figures 3** and **4**).

Effect of striatal DA depletion on GFAP, S100B, and STAT3 levels

The previous results indicated that striatal DA depletion induced morphological changes in GFAP-, S100B-, and STAT3-immunoreactive cells. Western blotting results revealed that GFAP protein expression was significantly higher in the 6-OHDA group than in controls ($P = 0.0015$, $n = 8$). Striatal DA depletion also induced an increase in the expression of S100B protein compared with controls ($P = 0.0001$, $n = 8$), and there was a significant increase in STAT3 protein levels in the 6-OHDA group compared with the control group ($P = 0.0002$, $n = 8$; **Figure 3C** and **D**).

Discussion

The present study demonstrated that astrocytes display a reactive phenotype following DA depletion. The most important pathological change observed in patients with PD is the degeneration and death of dopaminergic neurons in the substantia nigra of the midbrain, which significantly reduces DA levels in the striatum (Dauer and Przedborski, 2003). Astrocytes play an important role in this pathological process. Studies have shown that DA depletion in the striatum is associated with increased numbers of astrocytes (+20%), the astrocytic volume (+60%), and the numbers of glutamatergic synapses contacted by glutamate transporter 1 (GLT1)-positive astrocytic contacts (+15%) (Charron et al., 2014). In line with these results, we found that the size of GFAP-immunoreactive soma, the average optical densities of GFAP-immunoreactive cells, and the number of GFAP-immunoreactive cells were all significantly increased in 6-OHDA-treated rats. Moreover, western blotting results demonstrated that expression levels of GFAP were also significantly increased in 6-OHDA-treated rats. Increasing evidence has revealed that astrocytes undergo distinct changes in function and phenotype in the PD brain or in response to DA depletion. These altered astrocytes are known as reactive astrocytes. The presence of reactive astrocytes is a nonspecific but characteristic response that is associated with various changes in morphology and glial-derived factors. Our study suggests that, as the volume of striatal astrocytes increases, the number of somatic and protruding processes also increases. These changes are associated with DA depletion. Reactive astrocytes form a continuum, from subtle changes in gene expression to subtle changes in scar formation of molecules, cells, and functions. Astrocyte dysfunction is an important source of reactive astrocytes, and may be a potential mechanism that causes or participates in central nervous system disorders by decreasing normal astrocyte activities and/or increasing abnormal astrocyte activities. Astrocytes play an important role in synaptic physiology by controlling synapses, in part through K^+

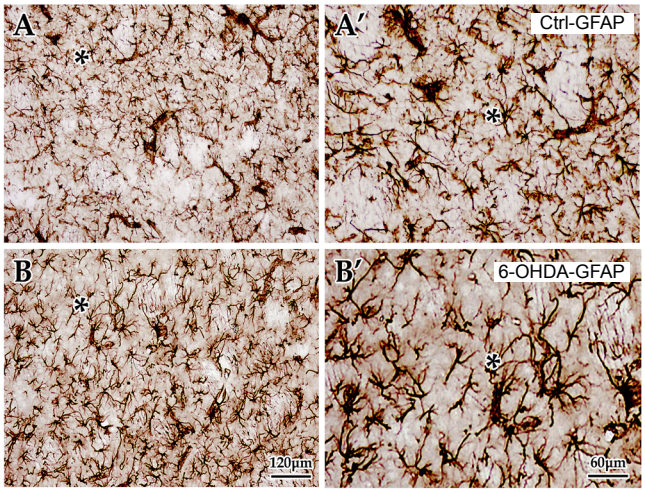

Figure 1 Effect of 6-OHDA on astrocytes in the striatum (immunohistochemical staining).
(A, A') Control group; (B, B') 6-OHDA group. (A', B') Enlargements of the * region of A and B, respectively. GFAP-immunoreactive cells displayed intense staining, thickened processes, and enlarged somas in the 6-OHDA group. Scale bars: 120 μm in A, B; 60 μm in A', B'. 6-OHDA: 6-Hydroxydopamine; Ctrl: control; GFAP: glial fibrillary astrocytic protein.

uptake and glutamic acid or glial-derived factors (Nedergaard and Verkhratsky, 2012). Therefore, it is necessary to explore changes in glial-derived factors in astrocytes during recurring deprivation.

Convincing evidence indicates that the danger-associated protein S100B plays an important role in PD (Schaf et al., 2005; Sathe et al., 2012; Fardell et al., 2018). Here, we show that S100B expression was increased in astrocytes after striatal DA innervation. Reactive gliosis after striatal dopaminergic innervation is positively correlated with S100B. S100B is a unique glial-derived factor that plays an important role in neuroinflammation and neurodegeneration (Rothermundt et al., 2003; Van Eldik and Wainwright, 2003). S100B is released from reactive astrocytes and activates RAGE to promote neuroinflammation in the brain (Esposito et al., 2008). Once released, S100B accumulates around the RAGE, and this interaction leads to JAK-STAT3 phosphorylation (Schulz et al., 2011). By activating this signaling pathway, S100B induces the transcription of proinflammatory cytokines. It is therefore conceivable that by specifically targeting the interaction of JAK-STAT in the brain, S100B-dependent neuroinflammation in PD may be inhibited. The present results indicated that not all astrocytes display the same GFAP–S100B and GFAP-STAT3 immunoreactivity responses to 6-OHDA-induced DA depletion. This result suggests that astrocyte-related protein expressions in the dorsolateral striatum react differently to dopaminergic denervation. Changes in STAT3 expression following striatal DA deprivation were further explored. After DA deprivation, reactive gliosis increased STAT3 expression. Our results suggest a correlation between STAT3 and astrocytic proliferation. Some studies have shown that STAT3 is the main signal transduction element in the inflammatory response, and that S100B is activated by JAK and then phosphorylates STAT3, inducing the

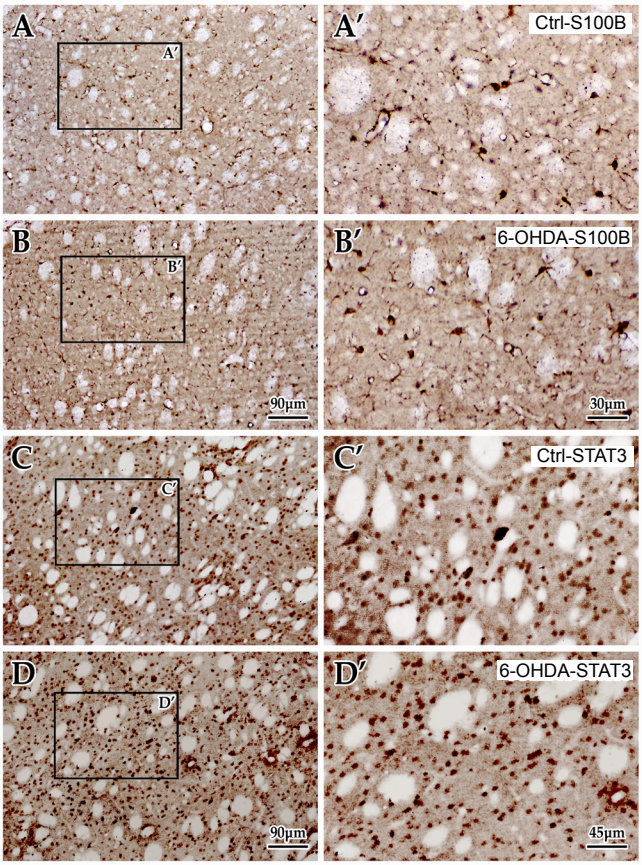

Figure 2 Immunohistochemistry of S100B and STAT3 in the striatum following dopamine depletion.
(A, A', C, C') Control group; (B, B', D, D') 6-OHDA group. (A'–D') Enlargement of the boxed region of A, B, C, and D, respectively. S100B-immunoreactive cells were uniformly distributed, with small somas and few processes (A, B). STAT3-immunoreactive cells were uniformly distributed in the striatum, and cell bodies were small with few processes (C', D'). Some of the larger STAT3-immunoreactive cells were observed to be interneurons, as determined by morphological characteristics of striatal cells (C', D'). Scale bars: 90 μm in A–D; 30 μm in A' and B'; 45 μm in C' and D'. 6-OHDA: 6-Hydroxydopamine; Ctrl: control; S100B: calcium-binding protein B; STAT3: signal transducer and activator of transcription 3.

translocation of STAT3 into the nucleus.

In conclusion, S100B and STAT3 may be involved in the striatal astrocyte activation that is induced by DA deprivation. This may be important for understanding the pathological mechanism of PD and for developing clinical treatments of this disease. However, further studies are needed to elucidate the mechanism of the correlation between STAT3 and S100B. This study confirms that S100B and STAT3 may be potential therapeutic targets of PD.

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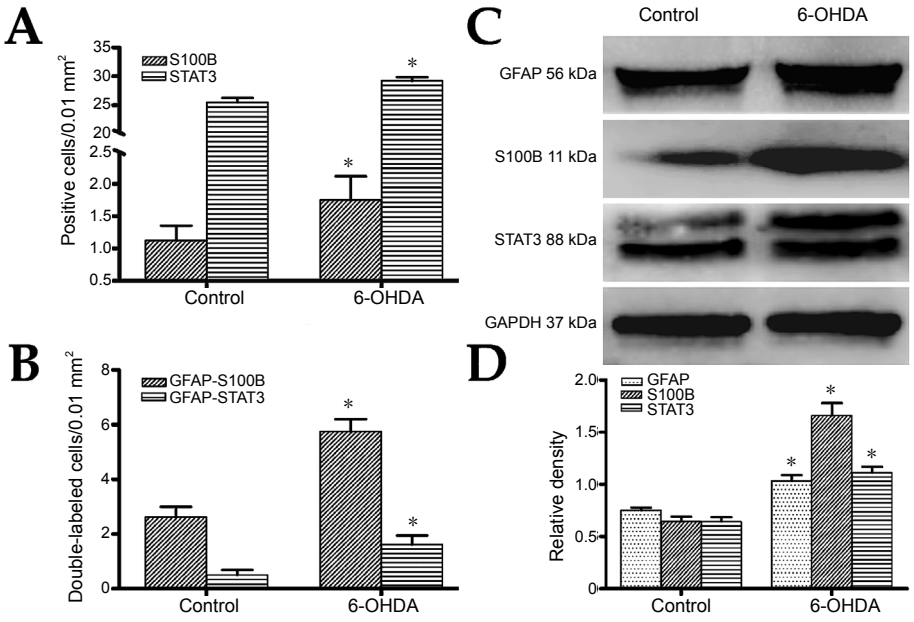

Figure 3 Effect of striatal dopamine depletion on GFAP, S100B, and STAT3 protein expression levels.
(A) S100B- and STAT3-immunoreactive cells were increased in the 6-OHDA group compared with the control group. (B) Quantitative results of immunofluorescence double-labeling of GFAP/S100B and GFAP/STAT3, which indicate that the number of both GFAP/S100B and GFAP/STAT3 coexpressing cells increased in the 6-OHDA group compared with the control group. (C) Protein levels of GFAP, S100B, and STAT3 in the striatum; GFAP, S10B and STAT3 protein levels were increased in the 6-OHDA group compared with the control group. $*P < 0.05$, *vs.* control group. 6-OHDA: 6-Hydroxydopamine; GFAP: glial fibrillary astrocytic protein; S100B: calcium-binding protein B; STAT3: signal transducer and activator of transcription 3.

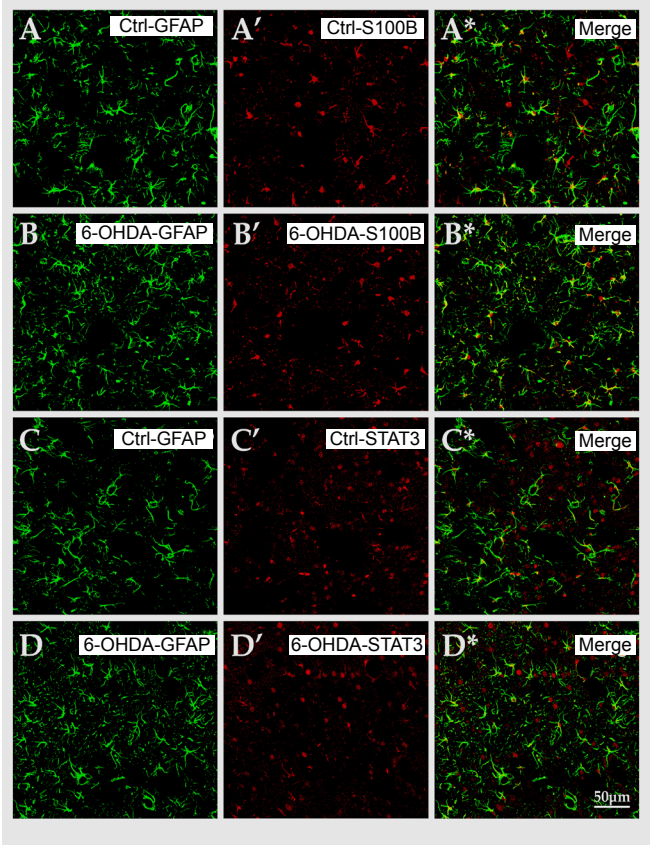

Figure 4 Coexpression of striatal astrocytes with S100B and STAT3 following dopamine depletion.
(A–D) GFAP-immunoreactive cells (green); (A', B') S100B-immunoreactive cells (red); (C', D') STAT3-immunoreactive cells (red); (A*, B*) the merged images (GFAP/S100B, yellow); (C*, D*) the merged images (GFAP/STAT3, yellow). (A, A', C, C') Control group; (B, B', D, D') 6-OHDA group. The number of cells coexpressing GFAP/S100B was higher in the 6-OHDA group (B*) than in the control group (A*). The number of GFAP/STAT3 double-labeled cells was also significantly increased in the 6-OHDA group (D*) compared with the control group (C*). Scale bar: 50 μm. 6-OHDA: 6-Hydroxydopamine; Ctrl: control; GFAP: glial fibrillary astrocytic protein; S100B: calcium-binding protein B; STAT3: signal transducer and activator of transcription 3.

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