A novel dual GLP-1 and GIP receptor agonist is neuroprotective in the MPTP mouse model of Parkinson's disease by increasing expression of BNDF

Chenhui Ji[1], Guo-Fang Xue[1], Cao Lijun[1], Peng Feng[1], Dongfang Li[1], Lin Li[3], Guanglai Li[1], Christian Hölscher [1,2]

1. Second hospital, Shanxi medical University, Taiyuan, PR China

2. Biomedical and Life Science, Faculty of Health and Medicine, Lancaster University, Lancaster LA1 4YQ, UK

3. Key Laboratory of Cellular Physiology, Shanxi Medical University, Taiyuan, PR China

short title: *DA is protective in the MPTP mouse model*

Brain Research, in press

Corresponding author:

Prof. Guanglai Li
Department of Neurology,
The Second Affiliated Hospital of Shanxi Medical University
No. 382, Wuyi Road,
Taiyuan, Shanxi Province, China
Tel.: +86-13703587193
Email: smuneu@163.com

Abstract

The incretins glucagon-like peptide 1 (GLP-1) and glucose dependent insulinotropic polypeptide (GIP) are growth factors with neuroprotective properties. GLP-1 mimetics are on the market as treatments for type 2 diabetes and are well tolerated. Both GLP-1 and GIP mimetics have shown neuroprotective properties in animal models of Parkinson's and Alzheimer's disease. In addition, the GLP-1 mimetic exendin-4 has shown protective effects in a clinical trial in Parkinson's disease (PD) patients. Novel GLP-1/GIP dual-agonist peptides have been developed and are tested in diabetic patients. Here we demonstrate the neuroprotective effects of a novel dual agonist (DA-JC1) in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) mouse model of PD. MPTP was injected once-daily (20mg/kg i.p.) for 7 days, and the dual agonist was injected 30 min later i.p. (50nmol/kg bw). The PI3k inhibitor LY294002 (0.6mg/kg i.v.) was co-injected in one group. DA-JC1 reduced or reversed most of the MPTP induced motor impairments in the rotarod and in a muscle strength test. The number of tyrosine hydroxylase (TH) positive neurons in the substantia nigra (SN) was reduced by MPTP and increased by DA-JC1. The ratio of anti-inflammatory Bcl-2 to pro-inflammatory BAX as well as the activation of the growth factor kinase Akt was reduced by MPTP and reversed by DA-JC1. The PI3k inhibitor had only limited effect on the DA-JC1 drug effect. Importantly, levels of the neuroprotective brain derived neurotropic factor (BDNF) were reduced by MPTP and enhanced by DA-JC1. The results demonstrate that DA-JC1 shows promise as a novel treatment for PD.

Keywords: insulin; apoptosis; incretins; growth factor; Akt; neurons

1. Introduction

Parkinson disease (PD) is the second most common neurodegenerative disease after Alzheimer disease, and current demographic trends indicate a life-time risk approaching 4% and predict a doubling of prevalence by 2030 (Schapira, 2013). It is characterized clinically by a variety of motor dysfunctions such as resting tremor, bradykinesia, rigidity and postural instability (Langston, 2002). These symptoms are attributed to the reduction in striatal dopamine (DA) level, which results from the selective and progressive degeneration of dopaminergic neurons in the substantia nigra pars compacta (SNpc) (Moore et al., 2005; Wakamatsu et al., 2008). Several risk factors have been identified, and type 2 diabetes is one of these (Hu et al., 2007; Schernhammer et al., 2011; Sun et al., 2012; Wahlqvist et al., 2012). Previous studies have documented the importance of insulin signaling in the brain (Freiherr et al., 2013; Ghasemi et al., 2013; van der Heide et al., 2006), and the fact that insulin signaling is compromised in the brains of patients with PD (Aviles-Olmos et al., 2013b; Moroo et al., 1994; Morris et al., 2011). In diabetes, analogues of incretin hormones have been developed to improve insulin signaling (Campbell and Drucker, 2013; Holst, 2004). The key incretin hormones are glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) (Baggio and Drucker, 2007; Campbell and Drucker, 2013). It has been confirmed that GLP-1 receptor agonists and GIP receptor agonists can pass through the blood brain barrier (Faivre and Holscher, 2013b; Hunter and Holscher, 2012; McClean and Holscher, 2014), protect neurons under oxidative stress, inhibit apoptosis, promoting neuronal proliferation and neuronal cells to grow new projections (Holscher, 2014b; JI et al., 2015; Li et al., 2010b; Li et al., 2015; Sharma et al., 2013). GLP-1 receptor agonists have shown protective effects in animal models of Alzheimer's disease (Bomfim et al., 2012; Li et al., 2010a; McClean et al., 2011), and clinical trials have started (Holscher, 2014a) with first positive results having been published (Gejl et al., 2015). GIP analogues also have shown protective effects in animal models of Alzheimer's disease (Duffy and Holscher, 2013; Faivre and Holscher, 2013a; Faivre and Holscher, 2013b).

Importantly, previous investigations found that GLP-1 receptor agonists also showed good neuroprotective effects in animal models of PD (Bertilsson et al., 2008; Harkavyi et al., 2008; Li et al., 2009; Liu et al., 2015; Zhang et al., 2015) and showed good effects in a pilot study in PD patients (Aviles-Olmos et al., 2013a; Aviles-Olmos et al., 2014).

The new drug DA-JC1, which is a dual- GLP-1/GIP receptor agonist (see materials and methods for the peptide sequence), shows superior effects in animal models of diabetes compared with liraglutide. This dual incretin agonist has been engineered to activate both GLP-1 and GIP receptors with comparable affinity, and demonstrated enhanced insulinotropic efficacy relative to single GLP-1 agonists (Finan et al., 2013). Some of these dual agonist peptides are already in clinical trials in patients with diabetes, and first results show good effects with fewer side effects compared to GLP-1 mimetics (Finan et al., 2013). We therefore tested the effects of a potent GLP-1/GIP receptor agonists in the MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropypridine) mouse model of PD. MPTP can selectively damage neurons in the nigrostriatal dopaminergic pathway and cause Parkinsonism in humans, nonhuman primates, and mice, mice have therefore become well accepted as a model for PD (Bove and Perier, 2012; Glover et al., 1986; Morin et al., 2014).

In a previous study, we have confirmed that the DA-JC1 has neuroprotective effects in MPTP-induced mice by increasing the number of TH, reducing the activation of astroglia and microglia (Cao et al., manuscript submitted). To further analyse the drug effects on neurodegenerative biomarkers in the brain of c57bl mice, we analysed the expression levels of brain derived neurotrophic factor (BDNF), a key neuroprotective growth factor (Kuipers and Bramham, 2006; Nagahara et al., 2013), and the expression of apoptosis signaling proteins (BAX, Bcl-2) (Sharma et al., 2013) using immunohistochemical and western blot methods. Moreover, we sought to determine whether neuroprotection by DA-JC1 against MPTP is mediated by the

activation of the PI3K/Akt pathway, a key growth factor second messenger pathway (Holscher, 2014b; Talbot et al., 2012).

2. Results

2.1 DA-JC1 improved the MPTP-induced impairments in motor coordination and in muscle strength

A One-way ANOVA found an overall difference between groups for Rotarod performance: ($F=38.7$; $P<0.001$) for muscle strength test: ($F=10.9$; $P<0.001$), followed by Fisher's Least Significant Difference test (LSD) post-hoc tests. DA-JC1 enhanced motor coordination of MPTP-treated animals as reflected in the time they were able to stay on the RotaRod and improved their muscle strength as see in a traction test ($P< 0.05$). Animals that had received treatment with MPTP showed significant impairments in motor coordination compared to the control animals that had received saline ($P<0.05$). Treatment of DA-JC1 significantly reversed the motor impairments induced by MPTP ($P < 0.05$). However, no significant difference was found between the MPTP + DA-JC1 and MPTP + DA-JC1 + LY294002 groups ($P>0.05$). Data are represented as mean ± SEM, n=10 per group. See Fig. 1.

2.2 Immunohistochemistry

2.2.1 DA-JC1 attenuated the loss of nigral TH-positive neurons induced by MPTP in the substantia nigra.

The protective effects of DA-JC1 on the dopaminergic neurons in the SN of mice treated with MPTP are shown in Fig. 2. A One-way ANOVA showed an overall difference between groups ($F=30.34$; $P<0.001$) followed by LSD-t post-hoc tests. There were significant reductions in the number of TH-positive cells in the SNpc for the MPTP group compared with the control group (36.83 ± 4.62, $P < 0.05$). With the treatment of DA-JC1, the number of TH-positive cells was significantly higher than

those in MPTP treated mice (48.50±5.32, P<0.05). Co-injecting the PI3k inhibitor LY294002 only reduced the DA-JC1 effect marginally (44.6±5, P<0.05 vs. controls) and did not reduce the neuroprotective effect of DA-JC1 on neurons in the substantia nigra. Data are represented as mean ± SEM, n=6 per group.

2.2.2 DA-JC1 attenuated the reduced expression of BAX and increased the expression of BCL-2 induced by MPTP in the substantia nigra and striatum.

A One-way ANOVA found significant differences for Bcl-2 numbers in the SN (F=27.91; P<0.001) and in the striatum (F=36.7; P<0.001) and for BAX in the SN (F=21.6; P<0.001) and in the striatum (F=19.43; P<0.001), followed by LSD post-hoc tests. The number of Bcl-2-positive cells for the MPTP group was reduced compared with the control group (P<0.05), and there was an increase in the number of BAX-positive cells for the MPTP group compared with the control group (P<0.05). After treatment with DA-JC1, the number of Bcl-2-positive cells was significantly higher than those in MPTP treated mice (P < 0.05) and the number of BAX-positive cells was significantly lower. Also, the Bcl-2-positive cells with the MPTP+DA-JC1+LY294002 treatment were significantly lower in number in the striatum than those in MPTP+DA-JC1 treated mice (P<0.05), but not in the s. nigra (though there was a trend). The BAX-positive cell numbers with the MPTP+DA-JC1+LY294002 treatment were significantly higher than those in MPTP+DA-JC1 treated mice (P < 0.05). Data are represented as mean ± SEM, n=6 per group. See figs 3 and 4.

2.2.3 DA-JC1 normalised the MPTP-induced reduction in brain derived neurotrophic factor (BDNF) positive neurons in the substantia nigra and striatum.

The protective effects of DA-JC1 on the neurons in the SN and striatum of mice treated with MPTP are shown in Fig. 5. A one-way ANOVA found an overall difference in the SN (F=31.6; P<0.001) and striatum groups (F=25.3; P<0.001) with LSD post-hoc tests. There were reduced numbers of BDNF-positive cells in the

MPTP group compared with the control group (P<0.05). With the treatment of DA-JC1, the number of BDNF-positive cells was significantly higher than those in MPTP treated mice (P < 0.05). Data are represented as mean ± SEM, n=6 per group.

2.3 Western blot analyses

2.3.1 Decrease of BDNF levels by MPTP

Western blot analysis was performed on protein levels from isolated midbrain (Fig. 6). MPTP treated mice had lower BDNF levels compared with the control group (P < 0.05). DA-JC1 showed only a non-significant elevation of BDNF expression (P >0.05). N=4.

2.3.2 DA-JC1 prevented the decrease of Akt (Ser473) phosphorylation induced by MPTP

We studied whether DA-JC1-mediated neuroprotection in PD mice involves an Akt-dependent pathway. The levels of the phosphorylated Akt at serine residue 473 of Akt (pAkt) were measured and normalised for the total Akt levels. Striatal Akt levels remained unchanged after MPTP lesions or DA-JC1 treatments. Compared with the controls, the levels of phospho-Akt (Ser473) were markedly decreased in MPTP-treated mice (P < 0.05). In comparison, treatment with DA-JC1 reversed the decline of phosphor-Akt (Ser473) compared with the MPTP group (P<0.05). Futhermore, the level of p-Akt was lower after treatment with LY294002 compared to the MPTP+DA-JC1 group (P<0.05), but higher than the MPTP group (P<0.05). See Fig. 6. N=4.

2.3.3 DA-JC1 reversed the decrease of the Bcl-2/BAX ratio induced by MPTP

Bcl-2/BAX ratio provides an indication of the activation of apoptotic signaling, Bcl-2 is an anti-apoptotic factor, whereas BAX is pro-apoptotic. The results show that MPTP treatment decreased this ratio, compared with the control group (P < 0.05). However, the decrease in Bcl-2/BAX ratio was reversed when treated with DA-JC1 (P < 0.01) compared with the MPTP group. Furthermore, the ratio was lower after

treatment with LY294002 compared to the MPTP+DA-JC1 group ($P<0.05$), but was higher than in the MPTP group ($P<0.05$), demonstrating an effect of the PI3k inhibitor. See Fig. 6. N=4.

3. Discussion

The results demonstrate that the protease-resistant dual GLP-1/GIP receptor analogue showed some protection from the impairments induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) treatment. MPTP is a commonly used chemical to induce a Parkinson-like state in rodents (Kopin and Markey, 1988; Morin et al., 2014; Nakamura and Vincent, 1986). Previously we have shown that the same GLP-1/GIP dual agonist has protective effects in a mouse model of stroke (Ling et al., manuscript submitted). Furthermore, in a study in the MPTP mouse model of PD, we showed that mice were clearly impaired in their spontaneous locomotion, sensory-motor coordination and muscle strength after MPTP treatment, and that treatment with the novel GLP-1/GIP dual agonist was able to prevent or reverse these effects to some degree. We also demonstrate a protection from a reduction in TH positive cells in the SN, a finding we could confirm in the present study. In our previous study, we also showed that the pro-inflammatory cytokine TNF-α was increased by MPTP and reduced by DA-JC1. Furthermore, the expression levels of synaptophysin, a marker for synapse numbers, was much reduced by the MPTP treatment, and DA-JC1 was able to partially prevent or reverse this effect (Cao et al., manuscript submitted). Here, we found that the expression of the dopamine synthetising enzyme tyrosine hydroxylase was much reduced by MPTP in the substantia nigra. This suggests that dopamine synthesis was compromised, which may be the cause for the observed motor impairments. We also show in the present study that levels of BDNF are much reduced by MPTP, and that DA-JC1 was able to reverse this reduction to some degree. BDNF is a key growth factor that has been shown to protect synapses from toxic influences (Cheng and Mattson, 1994). In a mouse model

of Alzheimer's disease, BDNF protected synapses and kept them functional (Blurton-Jones et al., 2009; Nagahara et al., 2013). BDNF has also shown neuroprotective effects in models of PD (Stahl et al., 2011) and is beneficial in PD (Allen et al., 2013; Frazzitta et al., 2014; He et al., 2013). The observation that DA-JC1 increases BDNF in the substantia nigra could explain the protective effects on neurons and synapses. We also showed in this study that the activation of the growth factor signaling kinase Akt is involved in the neuroprotective effects shown here. Akt is a key kinase in second messenger cell signaling pathways that activate cell repair, cell proliferation and energy utilisation. Growth factors such as insulin, IGF-1 or BDNF activate Akt, and so does GLP-1 (Erdogdu et al., 2010; Kimura et al., 2009; Li et al., 2010b; Racaniello et al., 2010). Impairments in Akt signaling is associated with an increased risk of developing PD (Xiromerisiou et al., 2008). Co-administration of the PI3k inhibitor LY294002 partly prevented the protective effect of DA-JC1 only to a small degree. This suggests that there are additional kinases or parallel cellular signaling pathways involved in the protective effects of DA-JC1. Other second messenger pathways have been shown to be involved, such as the Erk1/2 pathway (Sharma et al., 2013). Further research is required to identify additional kinases that play a role in the protective processes. Importantly, the growth factor signaling molecule Bcl-2 was enhanced by DA-JC1, and the apoptosis signaling molecule BAX was reduced. Previous studies have shown that GLP-1 receptor activation modulates the expression of these key signaling molecules to reduce apoptosis and enhance cell proliferation (Li et al., 2010b; Sharma et al., 2013; Wang et al., 2012). We also demonstrated in the MPTP mouse model that activation of the GLP-1 receptor enhances Bcl-2 and reduces BAX signaling (Liu et al., 2015). The results presented here are encouraging and demonstrate the potential of simultaneously activating the GLP-1 and GIP incretin receptors to reduce neurodegenerative processes in PD. GLP-1 analogues have shown neuroprotective effects in various diseases, such as Alzheimer's disease, Parkinson's disease, head trauma or stroke (Darsalia et al., 2012; Holscher, 2013; McClean and Holscher, 2014; Perry and Greig, 2004; Sato et al., 2013; Tweedie et al., 2013). Furthermore, two

previous studies showed good protection of MPTP treated mice using the GLP-1 agonist exendin-4 (Kim et al., 2009; Li et al., 2009). We also tested the novel GLP-1 receptor agonists liraglutide, lixisenatide and (Val8)GLP-1gluPAL in this model with good effects (Liu et al., 2015; Zhang et al., 2015). These preclinical results are of great importance, as the GLP-1 receptor agonist Exendin-4 has been tested in a pilot study in Parkinson's patients with promising first results (Aviles-Olmos et al., 2013a; Aviles-Olmos et al., 2014). A larger Phase II study is ongoing to verify the protective effect in PD patients. Exendin-4 and liraglutide are also currently tested in clinical trials in Alzheimer's disease (Holscher, 2014a). A recently completed pilot study showed good effects of liraglutide in preventing the progressive deterioration in brain activity and energy utilisation (Gejl et al., 2015).

GIP also has neuroprotective effects and protects cognition, synapse numbers, synaptic plasticity, and reduces inflammation in the brain (Duffy and Holscher, 2013; Faivre et al., 2011; Faivre and Holscher, 2013a; Faivre and Holscher, 2013b). We have previously shown good protective effects of the GIP analogue (dAla2)GIPgluPAL in the MPTP mouse model of PD (Li et al, manuscript submitted). It is therefore sensible to activate GLP-1 and GIP receptors simultaneously, which may show superior effects. Several dual agonists are being tested in diabetes, and first preclinical and clinical results show that GLP-1/GIP dual-agonists are superior in controlling diabetes compared to the GLP-1 analogue liraglutide (Finan et al., 2013). The present study is a proof of concept to demonstrate that these novel analogues also have great potential as a treatment for neurodegenerative disorders. However, the MPTP toxin induced animal model of PD has its limitations. The dual analogues will have to be tested in other animal models of PD that use different chemicals to induce PD- like symptoms such as 6-OHDA or LPS injection into the brain (Bertilsson et al., 2008; Harkavyi et al., 2008), and in transgenic mouse models that express human mutated genes that are known to induce Parkinson's disease (Bobela et al., 2014; Giraldez-Perez et al., 2014). Another important test to be conducted is to treat animals *after* inducing the PD lesion in order to assess the regenerative effect of the novel drugs. Furthermore, a direct comparison with other incretin mimetics will have to be made to show superiority

over these older drugs, similar to the tests that demonstrated that the dual-agonists are superior to the GLP-1 analogue liraglutide (Finan et al., 2013). As some of the novel dual-agonists are already in clinical tests to be developed as a treatment for type 2 diabetes (Finan et al., 2013), it would be straightforward to test these drugs in clinical trials in patients with PD.

4. Materials and methods

4.1. Reagents

The dual agonist DA-JC1 (Peptide Purity: 95.77%) was obtained from the Shanghai Qiangyao Biological Technology (Shanghai, China). The purity of the peptide was confirmed by reversed-phase HPLC and characterised using matrix assisted laser desorption/ionisation time of flight (MALDI-TOF) mass spectrometry. MPTP was obtained from Sigma-Aldrich (St Louis, MO, USA). LY294002 and rabbit anti-Tyrosine Hydroxylase (TH) Polyclonal antibody, Rabbit anti-Akt (total) antibody were obtained from Cell Signal Technology (China). Rabbit anti-phospho-Akt (Ser473) antibody were bought from Sigma. Rabbit anti-BDNF antibody, Rabbit anti-Bcl-2, Rabbit anti-BAX were purchased from Bioworld (St Louis, MN, USA). Horseradish peroxidase (HRP)-conjugated anti-rabbit antibodies were obtained from the Boster Institute of Biotechnology (Wuhan, China).

Peptide sequence of the GLP-1/GIP dual agonist DA-JC1 (Finan et al., 2013), peptide 18 in the Finan et al. paper:

Y**X**EGTFTSDYSIYLDKQAA**X**EFVNWLLAGGPSSGAPPPS**K**-NH2
X = aminoisobutyric acid; **K** = Lys-γE-C_{16} acyl

4.2 Animals and treatment protocol

Male C57BL/6mice (20–22g) were bought from the Academy of Military Medical Sciences (AMMS China), and were group-housed on a 12:12-hour light-dark cycle at 22°C with free access to food and water. The animals were divided into five groups

with 10 in each. The effects of DA-JC1 on MPTP-induced parkinsonism were studied in the following experimental groups:(A) Control (saline, i.p.); (B) DA-JC1 (50nmol/kg/day i.p.); (C) MPTP (20 mg/kg/day i.p.) ; (D) MPTP (20 mg/kg/day i.p.) + DA-JC1 (50nmol/kg/day i.p.); (E) MPTP (mg/kg i.p.) + DA-JC1 (50nmol/kg/day i.p.)+LY294002 (0.6mg/kg iv). Animals in groups B–E received DA-JC1 and/or MPTP daily for 7 consecutive days, DA-JC1 treatment was given 30min after the MPTP administration. In group E, LY294002 treatment was given 30min after the DA-JC1 injection. The work was approved by the ethics committee of Shanxi province. All animal procedures were performed in accordance to National Institute of Health (NIH) guideline (NIH publication NO. 85-23. Revised 1985). All efforts were made to minimise animal suffering and to reduce the number of animal used during experimental procedures.

4.3 Behavioral Tests

4.3.1 Rotarod performance

The mouse was placed onto a rotating rod with auto acceleration smoothly from 5 to 20 rpm over a period of 50s (YLS-4C, Academy of medical sciences in Shandong, China). The length of time the mouse was able to stay on the rotating rod was recorded. For this test, three trials were run for each mouse in a 30-min interval.

4.3.2 Traction Test

Muscle strength was assessed by a traction test as previously published (Luo et al., 2011). Mice were lifted onto a horizontal wire, which the mouse gripped by its forepaws. The mouse was scored as 3 for gripping the wire with both hind paws, 2 for gripping the wire with one hind paw, and 1 for not gripping the wire with either hind paw. The experiment was repeated three times for each animal.

4.4 Immunohistochemistry analysis

Animals were perfused intracardially with saline followed by cold 4% paraformaldehyde. Brains were removed and fixed in 4% PFA overnight. Brains were

then embedded in paraffin, and coronal sections of 5µm thickness were cut using a Leica microtome. Immunostaining techniques were used to assess the TH (1:200, Abcam), BDNF (1:100; Bioworld), apoptosis protein (anti-bax, anti-bcl2 1:200) in the s. nigra and striatum. The DAB staining was analysed with an Axio Scope 1 (Zeiss, Germany) microscope and photographed with a digital camera. Images were analysed with Image-Pro Plus 6.0 (Media Cybernetics, USA). Sampling was according to stereological rules, starting the cutting of sections at a random location and taking every 4^{th} section, and then analysing densities of staining using unbiased 2D dissectors that are superimposed on the images (Gengler et al., 2012). N=6 sections per brain were analysed, n=6 per group.

4.5 Western blot

For Western blot analysis, isolated tissues from the ventral midbrain were rinsed twice with cold saline and homogenized in an ice cold RIPA buffer (containing 1% Triton X-100, 0.1% SDS, 1% deoxycholate) and phenyl-methylsulfonyl fluoride (PMSF). Lysates were cleared by centrifugation at 12,000 rpm at 4 ◦ C for 10 min, and the total protein content in the supernatant was determined using a BCA Protein Assay Kit. Samples were then added with loading buffer to the same concentration, boiled and centrifuged. Each sample was applied to a sodium dodecyl sulfate (SDS) polyacrylamide (12%) gel for electrophoresis. The gel was run at 80 V for 30min, and 120 V for 1 h, and then electrophoretically transferred to polyvinylidene difluoride (PVDF) membranes at 150 V for 1 h. The membranes were blocked for 2 h with 5% BSA or 5%milk in Tris-buffered saline (TBS) containing 0.05% Tween-20 (TBST) at room temperature. They were then incubated at 4 ◦ C overnight with the respective primary antibodies: Rabbit anti-Akt (1:2000 Abcam), Rabbit anti-p-Akt (1:1000 CST), Rabbit anti-BDNF (1:500 Bioworld), Rabbit BCL-2 (1:500 Bioworld), Rabbit BAX (1:500 Bioworld). After washing three times in TBST for 5 min, membranes were incubated for 1 h at home temperature with anti-rabbit IgG-peroxidase conjugated (1:2000 Boster). Immunoreactivity was visualised by an Enhanced ChemiLuminescence (ECL) fluorescence detection system.

4.6 Statistical analysis

All values were displayed as means standard error (SEM). For statistical analysis, SPSS 17.0 was used. Repeated measures analysis of variance and one-way analysis of variance (ANOVA) with LSD tests were used for post-hoc analysis of the experimental data. A probability value of less than 0.05 was considered to be statistically significant.

Funding:
The research had been supported by a grant of the Cure Parkinson's Trust UK, and a grant to CH under the '100 Foreign Expert' of the Shanxi province government. The funding bodies had no involvement in the research or publication.
The authors do not declare a conflict of interest.

Authors' contributions
CJ, G-F X and CL conducted the experiments. PF, DL, LL, GL designed the experiments. CJ and CH analysed the data and wrote the manuscript.

5. References

Allen, S.J., Watson, J.J., Shoemark, D.K., Barua, N.U., Patel, N.K., 2013. GDNF, NGF and BDNF as therapeutic options for neurodegeneration. Pharmacol Ther. 138**,** 155-75.

Aviles-Olmos, I., Dickson, J., Kefalopoulou, Z., Djamshidian, A., Ell, P., Soderlund, T., Whitton, P., Wyse, R., Isaacs, T., Lees, A., Limousin, P., Foltynie, T., 2013a. Exenatide and the treatment of patients with Parkinson's disease. J Clin Invest. 123**,** 2730-6.

Aviles-Olmos, I., Limousin, P., Lees, A., Foltynie, T., 2013b. Parkinson's disease, insulin resistance and novel agents of neuroprotection. Brain. 136**,** 374-84.

Aviles-Olmos, I., Dickson, J., Kefalopoulou, Z., Djamshidian, A., Kahan, J., Fmedsci, P.E., Whitton, P., Wyse, R., Isaacs, T., Lees, A., Limousin, P., Foltynie, T., 2014. Motor and Cognitive Advantages Persist 12 Months After Exenatide Exposure in Parkinson's Disease. J Parkinsons Dis. 4**,** 337-344.

Baggio, L.L., Drucker, D.J., 2007. Biology of incretins: GLP-1 and GIP. Gastroenterology. 132**,** 2131-57.

Bertilsson, G., Patrone, C., Zachrisson, O., Andersson, A., Dannaeus, K., Heidrich, J., Kortesmaa, J., Mercer, A., Nielsen, E., Ronnholm, H., Wikstrom, L., 2008. Peptide hormone exendin-4 stimulates subventricular zone neurogenesis in the adult rodent brain and induces recovery in an animal model of Parkinson's disease. J Neurosci Res. 86**,** 326-38.

Blurton-Jones, M., Kitazawa, M., Martinez-Coria, H., Castello, N.A., Muller, F.J., Loring, J.F., Yamasaki, T.R., Poon, W.W., Green, K.N., Laferla, F.M., 2009. Neural stem cells improve cognition via BDNF in a transgenic model of Alzheimer disease. Proc Natl Acad Sci U S A. 106**,** 13594-9.

Bobela, W., Zheng, L., Schneider, B.L., 2014. Overview of mouse models of Parkinson's disease. Curr Protoc Mouse Biol. 4**,** 121-39.

Bomfim, T.R., Forny-Germano, L., Sathler, L.B., Brito-Moreira, J., Houzel, J.C., Decker, H., Silverman, M.A., Kazi, H., Melo, H.M., McClean, P.L., Holscher, C., Arnold, S.E., Talbot, K., Klein, W.L., Munoz, D.P., Ferreira, S.T., De Felice, F.G., 2012. An anti-diabetes agent protects the mouse brain from defective insulin signaling caused by Alzheimer's disease- associated Abeta oligomers. J Clin Invest. 122**,** 1339-53.

Bove, J., Perier, C., 2012. Neurotoxin-based models of Parkinson's disease. Neuroscience. 211**,** 51-76.

Campbell, J.E., Drucker, D.J., 2013. Pharmacology, physiology, and mechanisms of incretin hormone action. Cell Metab. 17**,** 819-37.

Cheng, B., Mattson, M.P., 1994. NT-3 and BDNF protect CNS neurons against metabolic/excitotoxic insults. Brain Res. 640**,** 56-67.

Darsalia, V., Mansouri, S., Ortsater, H., Olverling, A., Nozadze, N., Kappe, C., Iverfeldt, K., Tracy, L.M., Grankvist, N., Sjoholm, A., Patrone, C., 2012. Glucagon-like peptide-1 receptor activation reduces ischaemic brain damage following stroke in Type 2 diabetic rats. Clin Sci (Lond). 122**,** 473-83.

Duffy, A.M., Holscher, C., 2013. The incretin analogue D-Ala(2)GIP reduces plaque load, astrogliosis and oxidative stress in an APP/PS1 mouse model of Alzheimer's disease. Neuroscience. 228**,** 294-300.

Erdogdu, O., Nathanson, D., Sjoholm, A., Nystrom, T., Zhang, Q., 2010. Exendin-4 stimulates proliferation of human coronary artery endothelial cells through eNOS-, PKA- and PI3K/Akt-dependent pathways and requires GLP-1 receptor. Mol Cell Endocrinol. 325**,** 26-35.

Faivre, E., Gault, V.A., Thorens, B., Holscher, C., 2011. Glucose-dependent insulinotropic polypeptide receptor knockout mice are impaired in learning, synaptic plasticity, and neurogenesis. J Neurophysiol. 105**,** 1574-1580.

Faivre, E., Holscher, C., 2013a. D-Ala2GIP facilitated synaptic plasticity and reduces plaque load in aged wild type mice and in an Alzheimer's disease mouse model. J Alzheimers Dis. 35**,** 267-83.

Faivre, E., Holscher, C., 2013b. Neuroprotective effects of D-Ala2GIP on Alzheimer's disease biomarkers in an APP/PS1 mouse model. Alzheimers Res Ther. 5**,** 20-28.

Finan, B., Ma, T., Ottaway, N., Muller, T.D., Habegger, K.M., Heppner, K.M., Kirchner, H., Holland, J., Hembree, J., Raver, C., Lockie, S.H., Smiley, D.L., Gelfanov, V., Yang, B., Hofmann, S., Bruemmer, D., Drucker, D.J., Pfluger, P.T., Perez-Tilve, D., Gidda, J., Vignati, L., Zhang, L., Hauptman, J.B., Lau, M., Brecheisen, M., Uhles, S., Riboulet, W., Hainaut, E., Sebokova, E., Conde-Knape, K., Konkar, A., DiMarchi, R.D., Tschop, M.H., 2013. Unimolecular dual incretins maximize metabolic benefits in rodents, monkeys, and humans. Sci Transl Med. 5**,** 209ra151.

Frazzitta, G., Maestri, R., Ghilardi, M.F., Riboldazzi, G., Perini, M., Bertotti, G., Boveri, N., Buttini, S., Lombino, F.L., Uccellini, D., Turla, M., Pezzoli, G., Comi, C., 2014. Intensive rehabilitation increases BDNF serum levels in parkinsonian patients: a randomized study. Neurorehabil Neural Repair. 28**,** 163-8.

Freiherr, J., Hallschmid, M., Frey, W.H., 2nd, Brunner, Y.F., Chapman, C.D., Holscher, C., Craft, S., De

Felice, F.G., Benedict, C., 2013. Intranasal insulin as a treatment for Alzheimer's disease: a review of basic research and clinical evidence. CNS Drugs. 27**,** 505-14.

Gejl, M., Gjedde, A., Egefjord, L., Møller, A., Hansen, S.B., Vang, K., Rodell, A., Brændgaard, H., Gottrup, H., Schacht, A., Møller, N., Brock, B., Rungby, J., 2015. No Decline of Brain Glucose Metabolism in Alzheimer's Disease Patients Treated with Liraglutide. In American Diabetes Association conference, Boston, USA, abstr. 1309-P.

Gengler, S., McClean, P., McCurtin, R., Gault, V., Holscher, C., 2012. Val(8)GLP-1 rescues synaptic plasticity and reduces dense core plaques in APP/PS1 mice. Neurobiol Aging. 33**,** 265-276.

Ghasemi, R., Dargahi, L., Haeri, A., Moosavi, M., Mohamed, Z., Ahmadiani, A., 2013. Brain insulin dysregulation: implication for neurological and neuropsychiatric disorders. Mol Neurobiol. 47**,** 1045-65.

Giraldez-Perez, R., Antolin-Vallespin, M., Munoz, M., Sanchez-Capelo, A., 2014. Models of alpha-synuclein aggregation in Parkinson's disease. Acta Neuropathol Commun. 2**,** 176.

Glover, V., Gibb, C., Sandler, M., 1986. The role of MAO in MPTP toxicity--a review. J Neural Transm Suppl. 20**,** 65-76.

Harkavyi, A., Abuirmeileh, A., Lever, R., Kingsbury, A.E., Biggs, C.S., Whitton, P.S., 2008. Glucagon-like peptide 1 receptor stimulation reverses key deficits in distinct rodent models of Parkinson's disease. J Neuroinflammation. 5**,** 19(1-9).

He, Y.Y., Zhang, X.Y., Yung, W.H., Zhu, J.N., Wang, J.J., 2013. Role of BDNF in central motor structures and motor diseases. Mol Neurobiol. 48**,** 783-93.

Holscher, C., 2013. Central effects of GLP-1: new opportunities for treatments of neurodegenerative diseases. J Endocrinol. 221**,** 31-41.

Holscher, C., 2014a. The incretin hormones glucagonlike peptide 1 and glucose-dependent insulinotropic polypeptide are neuroprotective in mouse models of Alzheimer's disease. Alzheimers Dement. 10**,** S47-S54.

Holscher, C., 2014b. Insulin, incretins and other growth factors as potential novel treatments for Alzheimer's and Parkinson's diseases. Biochem Soc Trans. 42**,** 593-9.

Holst, J.J., 2004. Treatment of type 2 diabetes mellitus with agonists of the GLP-1 receptor or DPP-IV inhibitors. Expert Opin Emerg Drugs. 9**,** 155-66.

Hu, G., Jousilahti, P., Bidel, S., Antikainen, R., Tuomilehto, J., 2007. Type 2 diabetes and the risk of Parkinson's disease. Diabetes Care. 30**,** 842-7.

Hunter, K., Holscher, C., 2012. Drugs developed to treat diabetes, liraglutide and lixisenatide, cross the blood brain barrier and enhance neurogenesis. BMC Neurosci. 13**,** 33-38.

JI, C., Xue, G.F., LI, G., Li, D., Hölscher, C., 2015. Neuroprotective effects of glucose-dependent insulinotropic polypeptide in Alzheimer’s disease. Rev Neursci. in**,** press.

Kim, S., Moon, M., Park, S., 2009. Exendin-4 protects dopaminergic neurons by inhibition of microglial activation and matrix metalloproteinase-3 expression in an animal model of Parkinson's disease. J Endocrinol. 202**,** 431-9.

Kimura, R., Okouchi, M., Fujioka, H., Ichiyanagi, A., Ryuge, F., Mizuno, T., Imaeda, K., Okayama, N., Kamiya, Y., Asai, K., Joh, T., 2009. Glucagon-like peptide-1 (GLP-1) protects against methylglyoxal-induced PC12 cell apoptosis through the PI3K/Akt/mTOR/GCLc/redox signaling pathway. Neuroscience. 162**,** 1212-9.

Kopin, I.J., Markey, S.P., 1988. MPTP toxicity: implications for research in Parkinson's disease. Annu Rev Neurosci. 11**,** 81-96.

Kuipers, S.D., Bramham, C.R., 2006. Brain-derived neurotrophic factor mechanisms and function in adult synaptic plasticity: new insights and implications for therapy. Curr Opin Drug Discov Devel. 9**,** 580-6.

Langston, J.W., 2002. Parkinson's disease: current and future challenges. Neurotoxicology. 23**,** 443-50.

Li, Y., Perry, T., Kindy, M.S., Harvey, B.K., Tweedie, D., Holloway, H.W., Powers, K., Shen, H., Egan, J.M., Sambamurti, K., Brossi, A., Lahiri, D.K., Mattson, M.P., Hoffer, B.J., Wang, Y., Greig, N.H., 2009. GLP-1 receptor stimulation preserves primary cortical and dopaminergic neurons in cellular and rodent models of stroke and Parkinsonism. Proc Natl Acad Sci U S A. 106**,** 1285-90.

Li, Y., Duffy, K., Ottinger, M., Ray, B., Bailey, J., Holloway, H., Tweedie, D., Perry, T., Mattson, M., Kapogiannis, D., Sambamurti, K., Lahiri, D., Greig, N., 2010a. GLP-1 Receptor Stimulation Reduces Amyloid-beta Peptide Accumulation and Cytotoxicity in Cellular and Animal Models of Alzheimer's Disease. J Alzheimers Dis. 19**,** 1205-1219.

Li, Y., Tweedie, D., Mattson, M.P., Holloway, H.W., Greig, N.H., 2010b. Enhancing the GLP-1 receptor signaling pathway leads to proliferation and neuroprotection in human neuroblastoma cells. J Neurochem. 113**,** 1621-31.

Li, Y., Bader, M., Tamargo, I., Rubovitch, V., Tweedie, D., Pick, C.G., Greig, N.H., 2015. Liraglutide is neurotrophic and neuroprotective in neuronal cultures and mitigates mild traumatic brain injury in mice. J Neurochem. DOI: 10.1111/jnc.13169

Liu, W., Jalewa, J., Sharma, M., Li, G., Li, L., Hölscher, C., 2015. Neuroprotective effects of lixisenatide and liraglutide in the MPTP mouse model of Parkinson’s disease. Neuroscience. 303**,** 42-50.

Luo, F.C., Wang, S.D., Qi, L., Song, J.Y., Lv, T., Bai, J., 2011. Protective effect of panaxatriol saponins extracted from Panax notoginseng against MPTP-induced neurotoxicity in vivo. J Ethnopharmacol. 133**,** 448-53.

McClean, P., Parthsarathy, V., Faivre, E., Hölscher, C., 2011. The diabetes drug Liraglutide prevents degenerative processes in a mouse model of Alzheimer's disease. J Neurosci. 31**,** 6587-6594.

McClean, P.L., Holscher, C., 2014. Lixisenatide, a drug developed to treat type 2 diabetes, shows neuroprotective effects in a mouse model of Alzheimer's disease. Neuropharmacology. 86C**,** 241-258.

Moore, D.J., West, A.B., Dawson, V.L., Dawson, T.M., 2005. Molecular pathophysiology of Parkinson's disease. Annu Rev Neurosci. 28**,** 57-87.

Morin, N., Jourdain, V.A., Di Paolo, T., 2014. Modeling dyskinesia in animal models of Parkinson disease. Exp Neurol. 256**,** 105-16.

Moroo, I., Yamada, T., Makino, H., Tooyama, I., McGeer, P.L., McGeer, E.G., Hirayama, K., 1994. Loss of insulin receptor immunoreactivity from the substantia nigra pars compacta neurons in Parkinson's disease. Acta Neuropathol (Berl). 87**,** 343-8.

Morris, J.K., Bomhoff, G.L., Gorres, B.K., Davis, V.A., Kim, J., Lee, P.P., Brooks, W.M., Gerhardt, G.A., Geiger, P.C., Stanford, J.A., 2011. Insulin resistance impairs nigrostriatal dopamine function. Exp Neurol. 231**,** 171-80.

Nagahara, A.H., Mateling, M., Kovacs, I., Wang, L., Eggert, S., Rockenstein, E., Koo, E.H., Masliah, E., Tuszynski, M.H., 2013. Early BDNF treatment ameliorates cell loss in the entorhinal cortex of APP transgenic mice. J Neurosci. 33**,** 15596-602.

Nakamura, S., Vincent, S.R., 1986. Histochemistry of MPTP oxidation in the rat brain: sites of synthesis of the parkinsonism-inducing toxin MPP+. Neurosci Lett. 65**,** 321-5.

Perry, T.A., Greig, N.H., 2004. A new Alzheimer's disease interventive strategy: GLP-1. Curr Drug Targets. 5**,** 565-71.

Racaniello, M., Cardinale, A., Mollinari, C., D'Antuono, M., De Chiara, G., Tancredi, V., Merlo, D., 2010. Phosphorylation changes of CaMKII, ERK1/2, PKB/Akt kinases and CREB activation during early long-term potentiation at Schaffer collateral-CA1 mouse hippocampal synapses. Neurochem Res. 35**,** 239-46.

Sato, K., Kameda, M., Yasuhara, T., Agari, T., Baba, T., Wang, F., Shinko, A, Wakamori, T, Toyoshima, A, , Takeuchi, H., Sasaki, T., Sasada, S., Kondo, A, Borlongan, CV, Matsumae, M, Date, I, 2013. Neuroprotective effects of liraglutide for stroke model of rats. International journal of molecular sciences. 14**,** 21513-21524.

Schapira, A.H., 2013. Recent developments in biomarkers in Parkinson disease. Curr Opin Neurol. 26**,** 395-400.

Schernhammer, E., Hansen, J., Rugbjerg, K., Wermuth, L., Ritz, B., 2011. Diabetes and the risk of developing Parkinson's disease in Denmark. Diabetes Care. 34**,** 1102-8.

Sharma, M., Jalewa, J., Holscher, C., 2013. Neuroprotective and anti-apoptotic effects of Liraglutide on SH-SY5Y cells exposed to Methylglyoxal stress. J Neurochem. 128**,** 459-471.

Stahl, K., Mylonakou, M.N., Skare, O., Amiry-Moghaddam, M., Torp, R., 2011. Cytoprotective effects of growth factors: BDNF more potent than GDNF in an organotypic culture model of Parkinson's disease. Brain Res. 1378**,** 105-18.

Sun, Y., Chang, Y.H., Chen, H.F., Su, Y.H., Su, H.F., Li, C.Y., 2012. Risk of Parkinson disease onset in patients with diabetes: a 9-year population-based cohort study with age and sex stratifications. Diabetes Care. 35**,** 1047-9.

Talbot, K., Wang, H.Y., Kazi, H., Han, L.Y., Bakshi, K.P., Stucky, A., Fuino, R.L., Kawaguchi, K.R., Samoyedny, A.J., Wilson, R.S., Arvanitakis, Z., Schneider, J.A., Wolf, B.A., Bennett, D.A., Trojanowski, J.Q., Arnold, S.E., 2012. Demonstrated brain insulin resistance in Alzheimer's disease patients is associated with IGF-1 resistance, IRS-1 dysregulation, and cognitive decline. J Clin Invest. 122**,** 1316-38.

Tweedie, D., Rachmany, L., Rubovitch, V., Lehrmann, E., Zhang, Y., Becker, K.G., Perez, E., Miller, J., Hoffer, B.J., Greig, N.H., Pick, C.G., 2013. Exendin-4, a glucagon-like peptide-1 receptor agonist prevents mTBI-induced changes in hippocampus gene expression and memory deficits in mice. Exp Neurol. 239**,** 170-82.

van der Heide, L.P., Ramakers, G.M., Smidt, M.P., 2006. Insulin signaling in the central nervous system: learning to survive. Prog Neurobiol. 79**,** 205-21.

Wahlqvist, M.L., Lee, M.S., Hsu, C.C., Chuang, S.Y., Lee, J.T., Tsai, H.N., 2012. Metformin-inclusive sulfonylurea therapy reduces the risk of Parkinson's disease occurring with Type 2 diabetes in a Taiwanese population cohort. Parkinsonism Relat Disord. 18**,** 753-8.

Wakamatsu, M., Iwata, S., Funakoshi, T., Yoshimoto, M., 2008. Dopamine receptor agonists reverse behavioral abnormalities of alpha-synuclein transgenic mouse, a new model of Parkinson's disease. J Neurosci Res. 86**,** 640-6.

Wang, M.D., Huang, Y., Zhang, G.P., Mao, L., Xia, Y.P., Mei, Y.W., Hu, B., 2012. Exendin-4 improved rat cortical neuron survival under oxygen/glucose deprivation through PKA pathway. Neuroscience. 226**,** 388-96.

Xiromerisiou, G., Hadjigeorgiou, G.M., Papadimitriou, A., Katsarogiannis, E., Gourbali, V., Singleton, A.B., 2008. Association between AKT1 gene and Parkinson's disease: a protective haplotype.

Neurosci Lett. 436**,** 232-4.

Zhang, Y.F., Chen, Y.M., LI, L., Hölscher, C., 2015. Neuroprotective effects of (Val8)GLP-1-Glu-PAL in the MPTP Parkinson's disease mouse model. Behav Brain Res. 293**,** 107-113.

Figure captions

Fig. 1: Performance of mice in the RotaRod for 180 sec max. and muscle strength assessment is shown. *=P<0.05. A One-way ANOVA found an overall difference between groups for the Rotarod: (F=38.7; P<0.001) for muscle strength test: (F=10.9; P<0.001), followed by LSD-t post-hoc tests. Data are represented as mean ± SEM, n=10 per group.

Fig. 2: Quantification of TH- positive neurons in the substantia nigra pars compacta. A One-way ANOVA showed an overall difference between groups (F=30.34; P<0.001) followed by LSD-t post-hoc tests; *=P<0.05. Data are represented as mean ± SEM, n=6 per group. Representative images are shown. A=control; B=MPTP group; C=DA-JC1 group; D=MPTP+ DA-JC1 group; E=MPTP+ DA-JC1+Ly group. Scale bar = 250µm.

Fig. 3: Quantification of Bcl-2 positive neurons in the substantia nigra pars compacta and the striatum. A One-way ANOVA showed an overall difference between groups (P<0.001) followed by LSD-t post-hoc tests; *=P<0.05. Data are represented as mean ± SEM, n=6 per group. Representative images are shown. A, F=control; B, G=MPTP group; C, H=DA-JC1 group; D, I=MPTP+ DA-JC1 group; E, J= MPTP+ DA-JC1+ LY294002 group. Scale bar = 60µm.

Fig. 4: Quantification of BAX positive neurons in the substantia nigra pars compacta and the striatum. A One-way ANOVA showed an overall difference between groups (P<0.001) followed by LSD-t post-hoc tests; *=P<0.05. Data are represented as mean ± SEM, n=6 per group. Representative images are shown. A, F=control; B, G=MPTP group; C, H=DA-JC1 group; D, I=MPTP+ DA-JC1 group; E, J= MPTP+ DA-JC1+ LY294002 group. Scale bar = 60µm.

Fig. 5: Quantification of BDNF positive neurons in the substantia nigra pars compacta and the striatum. A One-way ANOVA showed an overall difference between groups (P<0.001) followed by LSD-t post-hoc tests; *=P<0.05. Data are represented as mean ± SEM, n=6 per group. Representative images are shown. A, E=control; B, F=MPTP group; C, G=DA-JC1 group; D, H=MPTP+ DA-JC1 group. Scale bar = 60µm.

Fig. 6: Western blot quantification of protein levels of Bcl-2, BAX, Akt, pAkt, and BDNF. A One-way ANOVA showed an overall difference between groups (P<0.001) followed by LSD-t post-hoc tests; *=P<0.05. Data are represented as mean ± SEM, Data are averages of 4 repetitions of blotting.

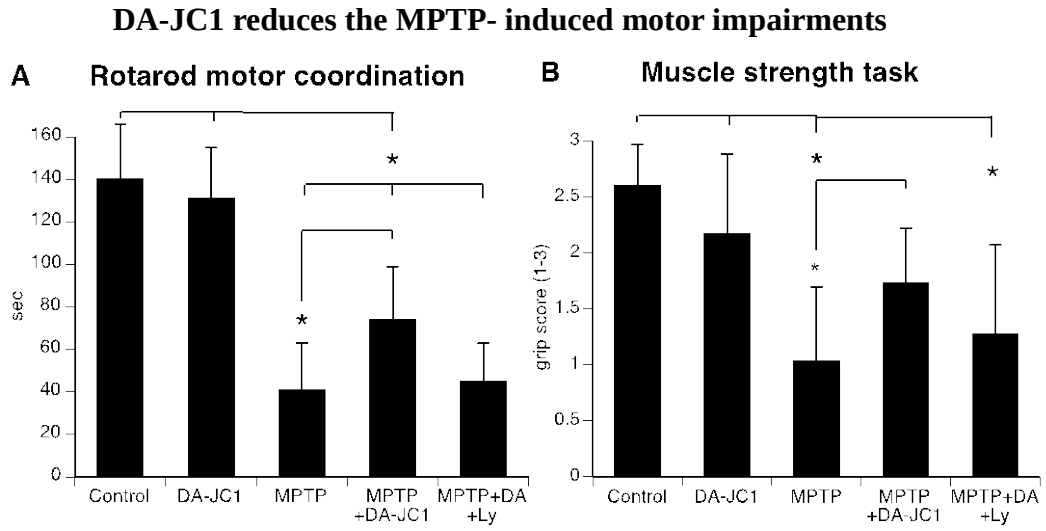
DA-JC1 reduces the MPTP- induced motor impairments
A
Rotarod motor coordination
sec
160
140
120
100
80
60
40
20
0
Control
DA-JC1
MPTP
MPTP +DA-JC1
MPTP+DA +Ly
B
Muscle strength task
grip score (1-3)
3
2.5
2
1.5
1
0.5
0
Control
DA-JC1
MPTP
MPTP +DA-JC1
MPTP+DA +Ly

Fig. 1

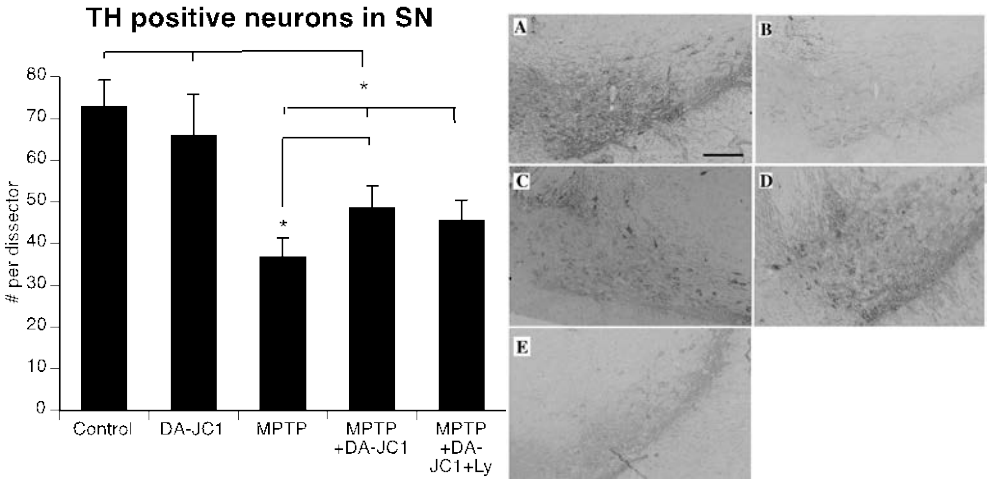
TH positive neurons in SN
per dissector
80
70
60
50
40
30
20
10
0
*
*
Control
DA-JC1
MPTP
MPTP +DA-JC1
MPTP +DA-JC1+Ly
A
B
C
D
E

Fig. 2

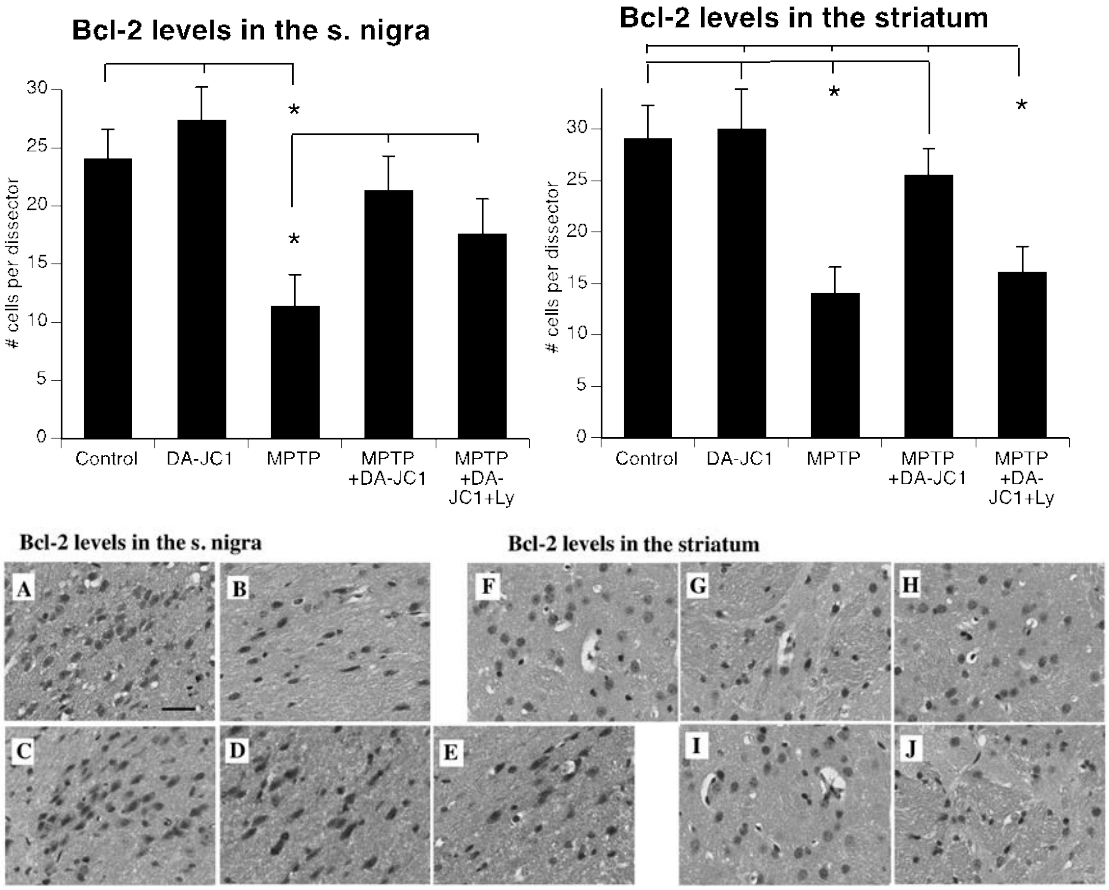
Bcl-2 levels in the s. nigra
cells per dissector
0
5
10
15
20
25
30
Control
DA-JC1
MPTP
MPTP +DA-JC1
MPTP +DA-JC1+Ly
*
*
Bcl-2 levels in the striatum
cells per dissector
0
5
10
15
20
25
30
Control
DA-JC1
MPTP
MPTP +DA-JC1
MPTP +DA-JC1+Ly
*
*
Bcl-2 levels in the s. nigra
Bcl-2 levels in the striatum
A
B
C
D
E
F
G
H
I
J

Fig 3

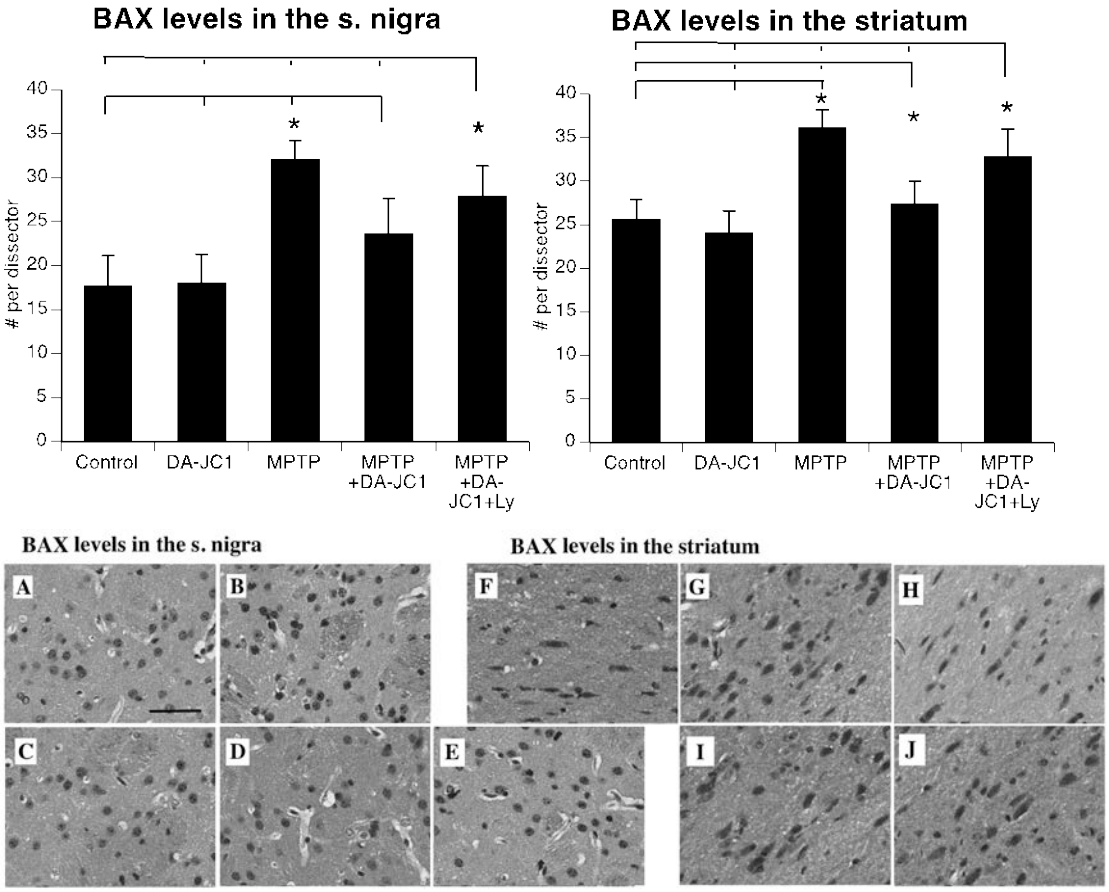
BAX levels in the s. nigra
per dissector
40
35
30
25
20
15
10
5
0
Control
DA-JC1
MPTP
MPTP +DA-JC1
MPTP +DA-JC1+Ly
BAX levels in the striatum
per dissector
Control
DA-JC1
MPTP
MPTP +DA-JC1
MPTP +DA-JC1+Ly
BAX levels in the s. nigra
BAX levels in the striatum
A
B
C
D
E
F
G
H
I
J

Fig. 4

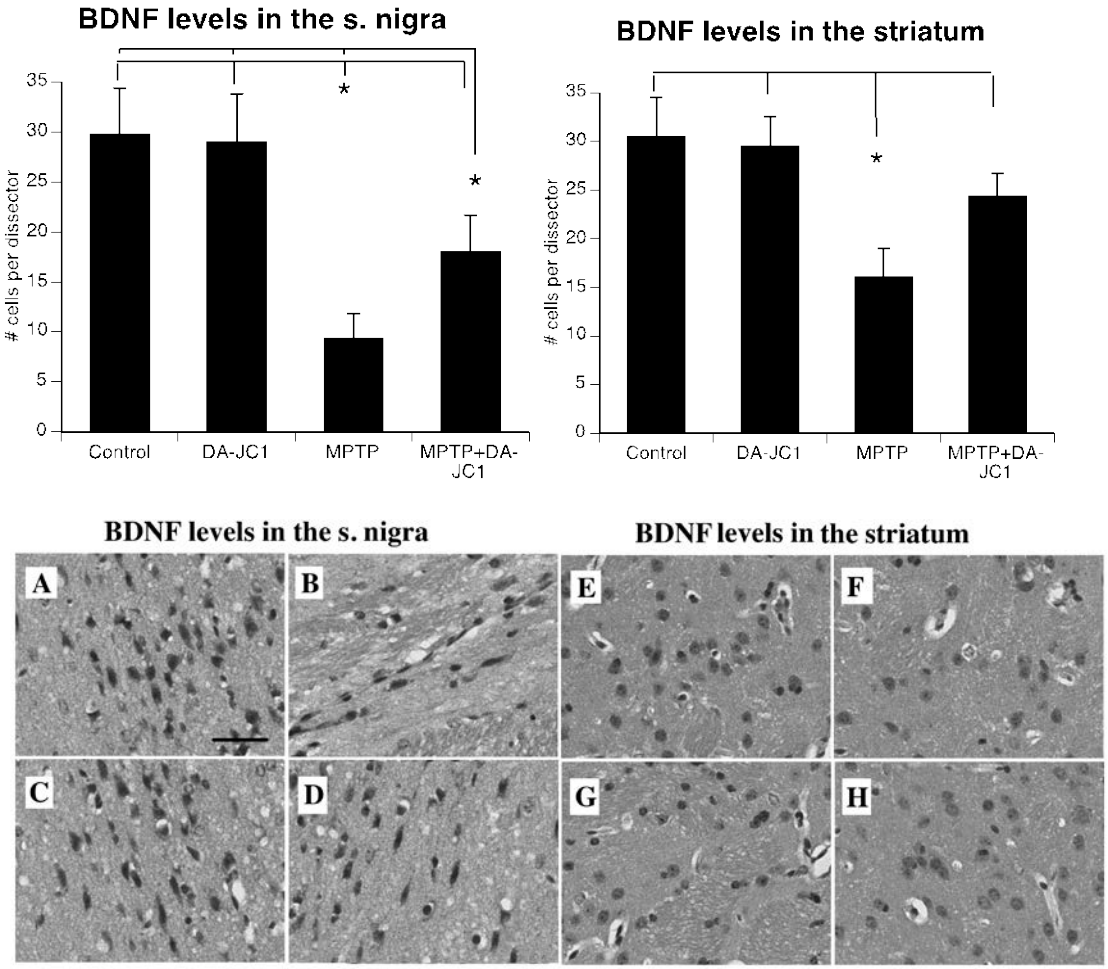
BDNF levels in the s. nigra
cells per dissector
35
30
25
20
15
10
5
0
Control
DA-JC1
MPTP
MPTP+DA-JC1
*
*
BDNF levels in the striatum
cells per dissector
35
30
25
20
15
10
5
0
Control
DA-JC1
MPTP
MPTP+DA-JC1
*
BDNF levels in the s. nigra
BDNF levels in the striatum
A
B
C
D
E
F
G
H

Fig. 5

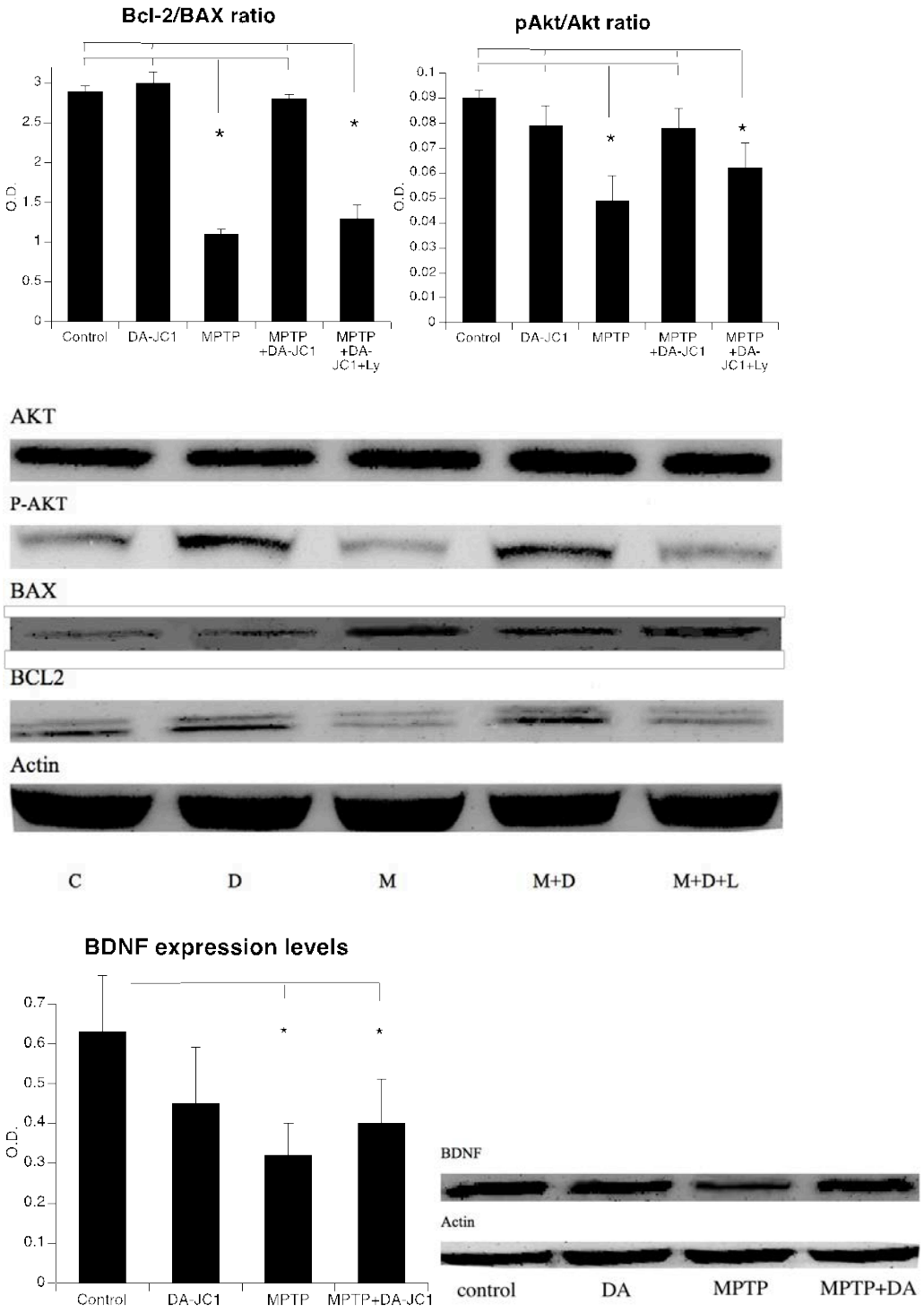
Bcl-2/BAX ratio
O.D.
Control
DA-JC1
MPTP
MPTP +DA-JC1
MPTP +DA-JC1+Ly
pAkt/Akt ratio
AKT
P-AKT
BAX
BCL2
Actin
C
D
M
M+D
M+D+L
BDNF expression levels
BDNF
Actin
control
DA
MPTP
MPTP+DA

Fig. 6