

INDIRECT APPLICATION OF NEAR INFRARED LIGHT INDUCES NEUROPROTECTION IN A MOUSE MODEL OF PARKINSONISM – AN ABSCOPAL NEUROPROTECTIVE EFFECT

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Abstract—We have previously shown near infrared light (Nlr), directed transcranially, mitigates the loss of dopaminergic cells in MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine)-treated mice, a model of parkinsonism. These findings complement others suggesting Nlr treatment protects against damage from various insults. However one puzzling feature of Nlr treatment is that unilateral exposure can lead to a bilateral healing response, suggesting Nlr may have ‘indirect’ protective effects. We investigated whether remote Nlr treatment is neuroprotective by administering different MPTP doses (50-, 75-, 100-mg/kg) to mice and treating with 670-nm light directed specifically at either the head or body. Our results show that, despite no direct irradiation of the damaged tissue, remote Nlr treatment produces a significant rescue of tyrosine hydroxylase-positive cells in the substantia nigra pars compacta at the milder MPTP dose of 50-mg/kg (~30% increase vs sham-treated MPTP mice, $p < 0.05$). However this protection did not appear as robust as that achieved by direct irradiation of the head (~50% increase vs sham-treated MPTP mice, $p < 0.001$). There was no quantifiable protective effect of Nlr at higher MPTP doses, irrespective of the delivery mode. Astrocyte and microglia cell numbers in substantia nigra pars compacta were not influenced by either mode of Nlr treatment. In summary, the findings suggest that treatment of a remote tissue with Nlr is sufficient to induce protection of the brain, reminiscent of the ‘abscopal effect’ sometimes observed in radiation treatment of metastatic cancer. This discovery has implications for the clinical translation of

light-based therapies, providing an improved mode of delivery over transcranial irradiation. © 2014 IBRO. Published by Elsevier Ltd. All rights reserved.

Key words: neuroprotection, near infrared light, MPTP, Parkinson’s disease, mouse model.

INTRODUCTION

There is now a substantial body of evidence, from animal models and cell culture studies, that photobiomodulation (PBM) with red to infrared light (600–1100 nm) has neuroprotective effects. Initially used as treatment to accelerate wound healing and recovery from soft tissue injury (Desmet et al., 2006; Huang et al., 2009), PBM has since been shown to protect photoreceptors from toxic or genetically-induced damage (Eells et al., 2003, 2008), reduce laser-induced retinal scarring (Eells et al., 2008) and mitigate brain pathology in animal models of Alzheimer’s disease (De Taboada et al., 2011; Grillo et al., 2013), traumatic brain injury (Oron et al., 2007; Xuan et al., 2013) and acute ischemic stroke (Oron et al., 2006). Findings in human patients suggest PBM yields therapeutic benefits in macular degeneration (Ivancic and Ivancic, 2008) and ischemic stroke (Lampl et al., 2007).

Much of our recent research has focused on whether PBM with near infrared light (Nlr; 670 nm) provides neuroprotection to mouse models of Parkinson’s disease. We have demonstrated that Nlr, delivered transcranially, mitigates dopaminergic cell loss in both acute and chronic MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) neurotoxin-induced models of parkinsonism (Shaw et al., 2010b; Peoples et al., 2012b; Moro et al., 2013) and a *tau* transgenic model of parkinsonism (Purushothuman et al., 2013). We have also demonstrated that Nlr partially corrects abnormal neuronal activity in the basal ganglia (Shaw et al., 2012) and improves motor behavior (Moro et al., 2013) in an acute MPTP model.

Although the neuroprotective efficacy of Nlr (and PBM generally) is now well established, the mechanism by which it protects CNS structures against degeneration remains elusive. The vast majority of investigators have focused on studying the direct effects of Nlr, at the cellular and tissue levels. There is strong evidence that

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Abbreviations: GFAP, glial fibrillary acidic protein; IBA1, ionized calcium-binding adapter molecule 1; LED, light-emitting device; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; Nlr, near infrared light; PBM, photobiomodulation; SNC, substantia nigra pars compacta; TH, tyrosine hydroxylase.

Nlr is absorbed by a key enzyme in the oxidative phosphorylation pathways of the mitochondrion, cytochrome c oxidase; Nlr-induced stimulation of cytochrome c oxidase increases electron transfer in the respiratory chain and results in an increase in ATP production and the generation of free radicals. This in turn triggers a cascade of secondary molecular events that assist the damaged cell, and tissue, to self-repair (Desmet et al., 2006; Huang et al., 2009; Rojas and Gonzalez-Lima, 2011; Chung et al., 2012).

The present study reports a test of an alternative idea: that treatment of a remote tissue with Nlr can induce systemic mechanisms that also provide 'indirect' protection of the brain. The evidence for such an indirect action is limited, because only a few studies have tested for or considered the possibility. In essence, these few studies have noted remote, often bilateral, effects on tissues, after local irradiation of skin wounds (Braverman et al., 1989), gliomas (implanted on the dorsum of mice, and irradiating abdomen) (Abe et al., 1993), skin abrasions (Hopkins et al., 2004) and oral mucosa lesions (Whelan et al., 2002). Further, recent studies have reported that critical tissues such as the brain, heart and lung are protected from stress by remote ischemic preconditioning (Kharbanda et al., 2009; Jensen et al., 2011). The stress involved in these conditioning regimes seems to elicit a protective response, despite the site of origin being limited to a remote part of the body (e.g. a limb), supporting the idea of systemic protective mechanisms.

As a first step to test whether the remote application of Nlr can protect the brain, we undertook a series of experiments to assess the neuroprotective efficacy, in MPTP-treated mice, of targeting Nlr specifically to the body, with no direct irradiation of the head. Preliminary results from this series of experiments have been published previously (Stone et al., 2013).

EXPERIMENTAL PROCEDURES

Ethics statement

All experiments were approved by the Animal Ethics Committee of University of Sydney (Approval Number: K03/6–2008/3/4792), and all efforts were made to minimize animal suffering.

MPTP injection regime

Following previous work (Shaw et al., 2010b, 2012), we used an acute MPTP mouse model. Male BALB/c mice ($n = 143$) were housed on a 12-h light/dark cycle with unlimited access to food and water. At 8 weeks of age, mice were injected intraperitoneally with either isotonic saline (control) or a dose of 25-mg/kg MPTP. Three different MPTP regimes were used: (1) two injections over 2 days (50-mg/kg total), (2) three injections over 3 days (75-mg/kg total) and (3) four injections over 4 days (100-mg/kg total).

Nlr treatment regime

One Nlr treatment consists of a 90-s exposure to 670-nm continuous wave light from a hand-held light-emitting device (LED, Quantum Devices WARP 10). The WARP 10 device delivers Nlr at a power density of 50-mW/cm², producing a dose of 4-J/cm² in a single exposure. Treatment was performed immediately following and 6 h after each injection. Two Nlr treatment paradigms were used: (1) mice were manually restrained by holding the body and Nlr light was directed at the head and (2) the head of the mouse was covered with infrared-opaque aluminum foil (to eliminate transcranial irradiation) and Nlr light was applied to the dorsum (Fig. 1). Sham treatment involved manually restraining and holding the mice under the LED device for 90 s but leaving the device turned off.

Measurement of Nlr transmittance in the substantia nigra pars compacta

To measure the level of Nlr transmittance to the substantia nigra pars compacta (SNc) during treatments with Nlr directed at the head or the body (with the head shielded as described above), a single mouse was positioned in a stereotactic frame and an optical fiber (0.39 NA 300- μ m diameter) was placed in the region of the SNc using stereotaxic coordinates (Paxinos and Franklin, 2001). The fiber was linked to a sensor (photodiode S120C + power meter PM100D; THORLABS, Newton, NJ, USA) or to a spectrometer (CCS200; THORLABS, Newton, NJ, USA) with integration detector. The mouse was then irradiated with Nlr from a WARP 10 LED, directed at either the head or the body (as described

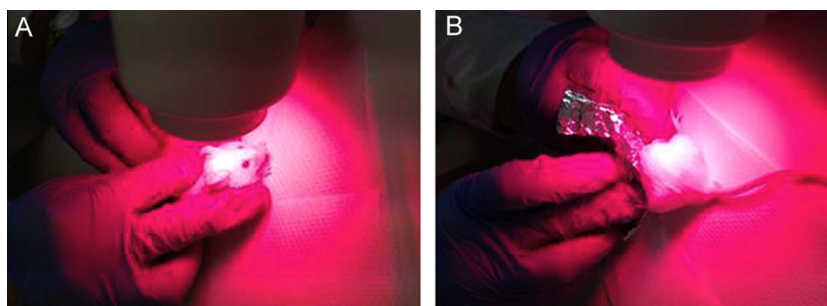


Fig. 1. Two different modes of Nlr delivery. Mice were restrained and treated with Nlr directed at (A) the head or (B) the body (dorsum), with the head shielded by aluminum foil.

in Nlr treatment regime). Each measurement was made at least three times on both sides of the brain.

Tissue processing and immunohistochemistry

Seven days after the final injection, mice were anesthetized with an intraperitoneal injection of sodium pentobarbital (60 mg/ml) and perfused transcardially with 4% buffered paraformaldehyde. Brains were post-fixed for 24 h in 4% paraformaldehyde and then cryoprotected in 30% sucrose/PBS. The midbrain was sectioned at 60 μm using a freezing microtome.

Sections were processed for routine tyrosine hydroxylase (TH) immunohistochemistry, as described previously (Shaw et al., 2010b). In addition, select sections were processed for immunohistochemistry using antibodies against glial fibrillary acidic protein (GFAP), a marker of astrocytes, or ionized calcium-binding adapter molecule 1 (IBA1), a marker of microglia. Briefly, free-floating sections were treated with 0.1% Triton in PBS for 1 h and then blocked in 10% normal goat serum in 0.1% Triton/PBS for 1 h. Sections were then incubated in either rabbit anti-TH (1:500; Sigma), rabbit anti-GFAP (1:500; DAKO) or rabbit anti-IBA1 (1:500; WAKO) at 4 °C for 3 days. This was followed by incubation in biotinylated anti-rabbit IgG (1:200; Bioscientific) for 4 h and then streptavidin–peroxidase complex (1:200; Bioscientific) for 2 h. Sections were then reacted in 3,3'-diaminobenzidine tetrahydrochloride (Sigma).

The number of labeled cells within the SNc was estimated using the optical fractionator method (Stereoinvestigator, MBF Science), as described previously (Shaw et al., 2010b, 2012; Peoples et al., 2012b). All analyses were conducted 'blinded', with the researcher undertaking the stereology having no prior knowledge of the experimental group from which a particular slide was taken. For comparisons between experimental groups, a one-way ANOVA test was performed, in conjunction with a Tukey multiple comparison test.

RESULTS

Transmittance of Nlr to the SNc

To confirm that our protocol for targeting Nlr to the body did not also lead to direct irradiation of the SNc, measurements of Nlr transmittance from the WARP 10 LED to the SNc were made. When Nlr was directed at the head of the mouse, signal was detected in the region of the SNc, however it had diminished considerably from the source; ~1% of the transmitted light intensity reached the SNc, which was approximately 5 mm from the light source. This result is consistent with previous measurements from our group (Shaw et al., 2010b; Moro et al., 2013) and others (Lapchak et al., 2004; Zivin et al., 2009). In contrast, when Nlr was directed at the body of the mouse, with the head region shielded with aluminum foil, we did not detect any signal in the SNc. This finding is also consistent with previous studies reporting that Nlr levels are not traceable more than ~20 mm away from the source; in our case, the SNc was well over 20 mm away from where Nlr was

applied to the body. In addition, our measurements of the penetration of Nlr directed at the body of the mouse suggest that ~15% of the transmitted light intensity penetrates the skin and fur to reach underlying body organs and tissues.

Nlr has no effect on TH⁺ cell number in control mice

The experiment involved two control groups, one receiving saline injections and sham treatments ($n = 17$) and another receiving saline injections and Nlr treatments ($n = 11$). Consistent with our observations in previous studies (Shaw et al., 2010b; Peoples et al., 2012b; Moro et al., 2013), assessment of TH⁺ cell counts from these two groups revealed very similar mean numbers and no significant difference ($p = 0.72$). Therefore, these two groups were combined for all subsequent statistical analyses.

Quantitative evaluation of the neuroprotective efficacy of Nlr treatment

TH immunohistochemistry was used to assess the impact of different acute MPTP regimes on dopaminergic cells of the SNc and evaluate the efficacy of different Nlr delivery modes in protecting against this insult. Three MPTP doses were used: (i) 50-mg/kg ($n = 58$), (ii) 75-mg/kg ($n = 24$) and (iii) 100-mg/kg ($n = 33$). Each dose was coupled with each of three different treatment approaches: (i) sham (i.e. no Nlr; $n = 8$ –36 per group), (ii) Nlr directed at the body (with the head shielded, $n = 8$ –10 per group) and (iii) Nlr directed at the head ($n = 8$ –17 per group) Table 1.

Fig. 2 shows TH⁺ cell counts in the SNc for each of these experimental groups, relative to mean TH⁺ cell number in the saline-injected control group (dotted line). For each of the different MPTP regimes, separate one-way ANOVAs were performed to identify variations across the different experimental groups (including the control group). For all MPTP doses, variations in TH⁺ cell counts between the groups were significant ($p < 0.0001$).

Relative to saline-injected controls, sham-treated MPTP mice showed a significant reduction in TH⁺ cell number at all MPTP doses (all $p < 0.001$). There did not appear to be a dose-dependent effect on loss of TH⁺ cells from the SNc, with all MPTP doses resulting in a similar reduction (~35–40%).

However MPTP dose did appear to impact on the capacity of Nlr treatment to provide neuroprotection. Mice receiving the milder MPTP dose (50-mg/kg) showed significantly higher TH⁺ cell counts when treated with Nlr directed at either the head (~50% higher, $p < 0.001$) or body (~30% higher, $p < 0.05$) than when sham treated. The TH⁺ cell counts in MPTP mice receiving Nlr to either the head or body did not differ significantly from saline-treated control levels ($p > 0.05$).

With a higher MPTP dose of 75-mg/kg, Nlr treatment directed at the body induced no significant rescue of TH⁺ cells in the SNc. Nlr treatment directed at the head induced a mean increase in TH⁺ cell number relative to

Table 1. TH⁺ cell number in the SNc following different doses of MPTP and Nir treatments

MPTP dose	Nlr regime		
	Sham	Nlr-Body	Nlr-Head
0 (control)	12,986 ± 526 (17)		12,694 ± 562 (11)
50	7976 ± 418 (36)	10,481 ± 1348 (11)	11,756 ± 484 (12)
% Bleached	58%	36%	17%
75	8189 ± 1198 (8)	8252 ± 1139 (8)	10,989 ± 911 (8)
% Bleached	50%	50%	17%
100	7484 ± 272 (9)	7530 ± 719 (9)	8094 ± 479 (19)
% Bleached	100%	77%	68%

Data are presented as mean cell count ± SEM. Group sizes are given in parentheses. '% Bleached' refers to the percentage of mice from each group that showed a 'bleached' appearance in TH-labeled sections of the SNc (equivalent to < 8500 TH⁺ cells).

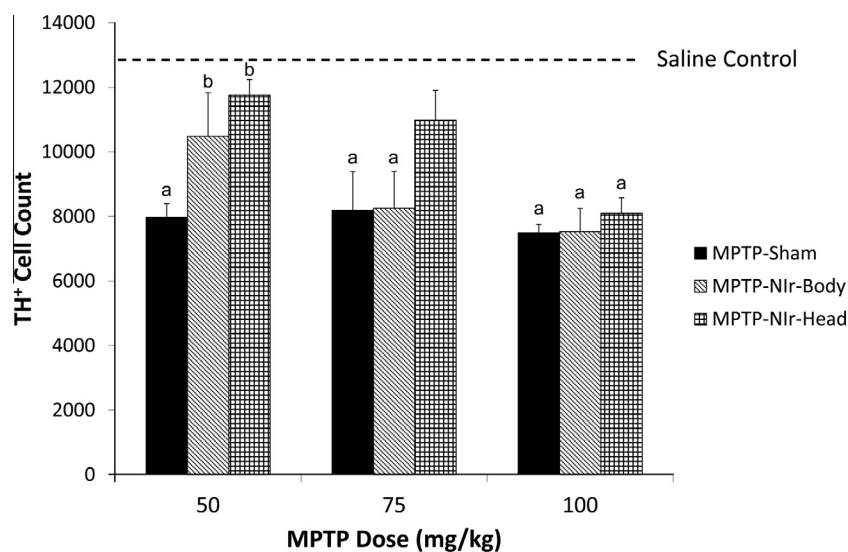


Fig. 2. TH⁺ cell number in the SNc following different doses of MPTP and Nir treatments. Data are presented as group mean ± SEM. The dotted line denotes the mean TH⁺ cell number for the saline-injected control group. ^a*p* < 0.05 vs saline control group, ^b*p* < 0.05 vs MPTP-Sham group. *n* = 8–36 per group.

sham-treated MPTP mice of ~35%, however this did not reach statistical significance using the Tukey post hoc test (*p* > 0.05).

With a still higher dose of MPTP (100-mg/kg), Nir treatment provided no rescue of TH⁺ cells, wherever directed. Regardless of whether Nir was directed at the head or the body, TH⁺ cell counts were significantly lower than saline-injected controls.

Qualitative evaluation of the neuroprotective efficacy of Nir treatment

The patterns described above are illustrated in Fig. 3. Relative to saline-injected controls (Fig. 3A,B), sham-treated MPTP groups (Fig. 3D,G,J) had fewer TH⁺ cells in the SNc; labeling was noticeably higher in both Nir-treated groups at the 50-mg/kg MPTP dose (Fig. 3E,F) and in the head-directed Nir-treatment group at the 75-mg/kg MPTP dose (Fig. 3I). No qualitative differences in TH labeling were observed with Nir treatment to the body at MPTP doses of 75- and 100-mg/kg (Fig. 3H,K) or Nir treatment to the head at 100-mg/kg MPTP (Fig. 3L).

A key sign of an MPTP-induced lesion is that the SNc has a 'bleached' or 'washed-out' appearance – a zone with few scattered TH⁺ cells. In our experience, not all mice receiving MPTP have this appearance, with some showing little histological evidence of MPTP toxicity. In the sham-treated MPTP groups given doses of 50- and 75-mg/kg, this bleached appearance was a feature of the SNc regions examined in 50–60% of the mice; the remaining mice appeared similar to controls (i.e. no bleached appearance). In the sham-treated MPTP group of the 100-mg/kg regime, all of the mice had a bleached SNc appearance Table 1.

The proportion of mice manifesting this bleached SNc decreased with Nir treatment, particularly at the milder MPTP doses (50- and 75-mg/kg), reflecting the quantitative patterns outlined above. For mice treated with Nir to the body, 35% of mice in the 50-mg/kg, 50% of mice in the 75-mg/kg and 80% of mice in the 100-mg/kg regimes had a bleached SNc appearance. For mice treated with Nir to the head, SNc bleaching was evident in 15% of mice in both the 50- and 75-mg/kg regimes and 70% of mice in the 100-mg/kg regime Table 1. It should be noted that all mice were included in the

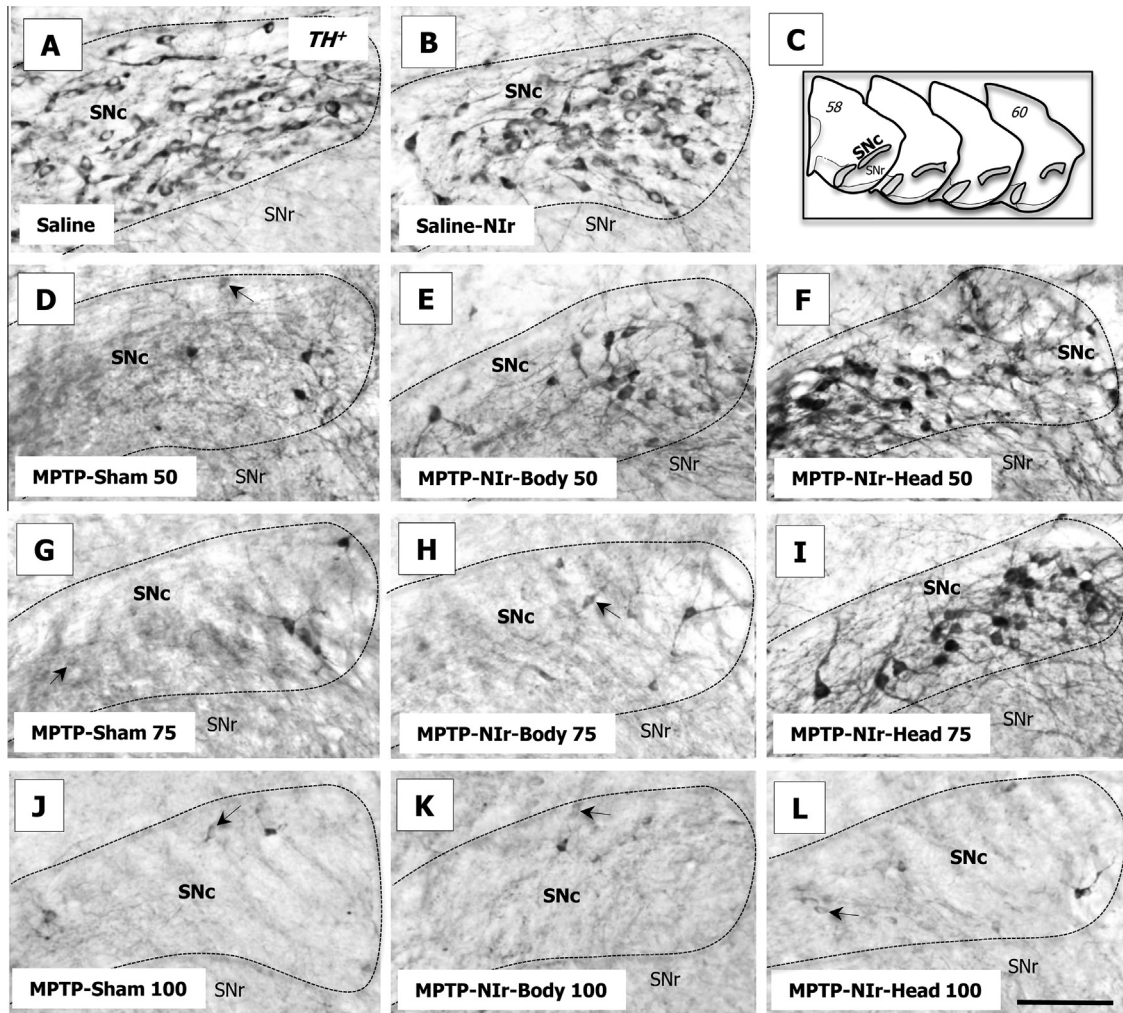


Fig. 3. Photomicrographs of TH⁺ cells in the SNc following different doses of MPTP and Nlr treatments. The different experimental groups shown are (A) Saline-Sham, (B) Saline-Nlr, (D, G, J) MPTP-Sham (50-, 75-, and 100-mg/kg) are indicated for each of the MPTP groups. (C) Schematic diagrams of coronal sections, across the rostrocaudal axis (from left to right; plates 58–60 of the mouse brain atlas (Paxinos and Franklin, 2001)), from where the photomicrographs were taken (lateral regions; shaded). Arrows indicate lightly labeled cells seen often in the MPTP groups. Many of the animals in the MPTP-Sham groups had a SNc that appeared washed-out or bleached; this appearance was less common in the MPTP-Nlr-Head groups of the 50- and 75-mg/kg regimes and the MPTP-Nlr-Body group of the 50-mg/kg regime. Nuclear boundaries were marked by following a light 'shadow' of TH immunoreactivity which defined the SNc. All figures are of coronal sections; dorsal to top and lateral to right. Scale bar = 100 μ m.

quantitative statistical analysis described above, whether bleached or not.

The role of glia in Nlr-induced neuroprotection

To gain insights into the glial response to Nlr treatment and whether glia may be involved in mediating Nlr-induced neuroprotection, we labeled SNc sections from the 75-mg/kg MPTP series for GFAP, a marker of astrocytes, and IBA1, a marker of microglia. Specifically, we selected sections from sham-treated MPTP animals that had a bleached TH⁺ appearance, and sections from Nlr-treated MPTP animals that showed some rescue of TH⁺ cell number.

Although there were increases in the mean number of GFAP⁺ cells in all MPTP groups relative to the saline control group, these increases did not reach statistical

significance; in fact, there were no significant differences in the number of GFAP⁺ cells across the different groups ($p = 0.21$; Fig. 4 Table 2). In contrast, variations in IBA1⁺ cell counts between the groups were significant ($p = 0.0002$). Mice receiving MPTP showed significantly higher IBA1⁺ cell counts in the SNc than saline-injected controls ($\sim 40\%$, $p < 0.01$). However this was not mitigated by Nlr treatment to either the head or body, with IBA1⁺ cell counts in these two groups similar to those in sham-treated MPTP mice, and significantly higher than in saline-injected controls ($p < 0.01$; Fig. 4 Table 2). Representative photomicrographs of GFAP and IBA1 labeling in the SNc of the different experimental groups are shown in Fig. 5. The GFAP and IBA1 labeling patterns observed following MPTP doses of 50- and 100-mg/kg were similar to those seen with the 75-mg/kg MPTP regime in Fig. 5 (data not shown).

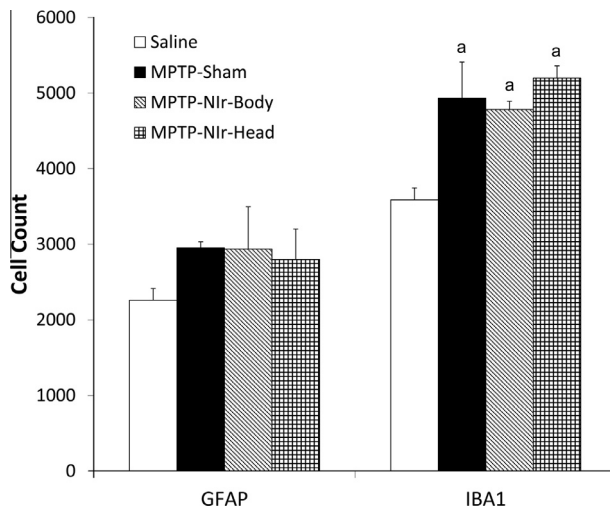


Fig. 4. Glial cell number in the SNc following 75-mg/kg MPTP with and without Nlr treatment. To assess glial cell activation or invasion in the SNc, counts were made of cells labeling positive for GFAP (a marker of astrocytes) or IBA1 (a marker of microglia). Data are presented as group mean \pm SEM. ^a $p < 0.05$ vs saline control group. $n = 3$ –6 per group for GFAP, $n = 4$ –9 per group for IBA1.

DISCUSSION

Our working hypothesis in recent studies (Shaw et al., 2010b, 2012; Peoples et al., 2012a,b; Moro et al., 2013; Purushothuman et al., 2013) has been that Nlr acts to protect midbrain dopaminergic cells by penetrating the cranium and the parenchyma of the brain to reach the midbrain, where it is absorbed by photoacceptors in the mitochondria of the dopaminergic cells, leading to the up-regulation of protective pathways in stressed cells. The findings of the present study provide support for a novel additional hypothesis – that Nlr, when applied to remote tissues, can have a protective effect on the brain.

Using different doses of MPTP, we have shown that remote Nlr is capable of rescuing dopaminergic neurons of the SNc from mild MPTP insult (50-mg/kg). This protective effect is diminished at stronger MPTP doses (75 and 100-mg/kg). Relative to direct Nlr irradiation of the head, remote Nlr does not appear to provide as robust a protection; for example, at a moderate MPTP dose of 75-mg/kg, Nlr targeting the head is likely to provide some protection of midbrain dopaminergic neurons, whereas there is no detectable protective effect of remote Nlr at this MPTP dose. Thus, although the overall number of TH⁺ cells did not vary between the different MPTP doses regimes, the stronger doses did reduce the likelihood of neuroprotection. The dopaminergic cells in the stronger dose regimes –

despite the fact that they still expressed TH – may have been more damaged intracellularly than with the milder dose, perhaps beyond the stage of repair (Jackson-Lewis et al., 1995; Bjorklund et al., 1997). These findings are consistent with the idea that neuroprotection is more likely when there is less toxic insult and/or prior neurodegeneration (Ashkan et al., 2007; Wallace et al., 2007). We did not observe any evidence of microglial or astrocyte involvement in mediating Nlr-induced neuroprotection, at least at the time point used in this study (7 days post-injection). Although MPTP was associated with an increase in IBA1 labeling in the SNc, presumably reflecting microglial invasion, this effect was not mitigated by Nlr treatment. However we cannot rule out the possibility that glia are involved in mediating earlier stages of cell rescue.

It should be noted that, while TH immunohistochemistry has been widely used to assess patterns of neurodegeneration and neuroprotection in models of parkinsonism (Wallace et al., 2007; Ma et al., 2009; Shaw et al., 2010a,b), it remains possible that the Nlr-induced mitigation of TH⁺ cell number we observed may reflect preservation of TH expression rather than preservation of cell survival (i.e. “true” neuroprotection). While there are reports of transient cellular TH expression in SNc (Paul et al., 2004), caudate putamen (Huot et al., 2007) and retina (Tatton et al., 1990) following parkinsonian insult, we believe that the majority of the Nlr-induced preservation of TH⁺ cell number reflects overall cell survival. Previous studies have delineated the sequence of events following MPTP insult; there is an initial impact on TH expression which, after a longer period, manifests as cell death (Bjorklund et al., 1997), as indicated by fewer Nissl-stained (and TH⁺) cells in the SNc of mice (Ma et al., 2009) and monkeys (Wallace et al., 2007). In any case, whether through effects on TH expression or cell survival, a key outcome of this study was that Nlr treatment was able to rescue TH expression, and presumably normal dopaminergic cell function, during a highly vulnerable period following MPTP insult (Peoples et al., 2012b).

Our results are suggestive of two different mechanisms by which Nlr can induce neuroprotection: direct stimulation of the damaged cells and indirect stimulation of as yet unidentified circulating mediators that transduce protective effects to the brain (Johnstone et al., 2014).

As noted in the Introduction, the concept that treatments localized to one area of the body can induce beneficial effects in other remote tissues is not novel. A similar phenomenon is observed in remote ischemic preconditioning, where the induction of ischemia in one tissue can elicit protection of other remote tissues to

Table 2. Glial cell number in the SNc following 75-mg/kg MPTP with and without Nlr treatment

Marker	MPTP-Nlr regime			
	Control	MPTP-Sham	MPTP-Nlr-Body	MPTP-Nlr-Head
GFAP	2256 \pm 156 (6)	2951 \pm 80 (4)	2935 \pm 561 (3)	2797 \pm 404 (3)
IBA1	3587 \pm 158 (9)	4933 \pm 477 (4)	4783 \pm 109 (4)	5198 \pm 161 (4)

Data are presented as mean cell count \pm SEM. Group sizes are given in parentheses.

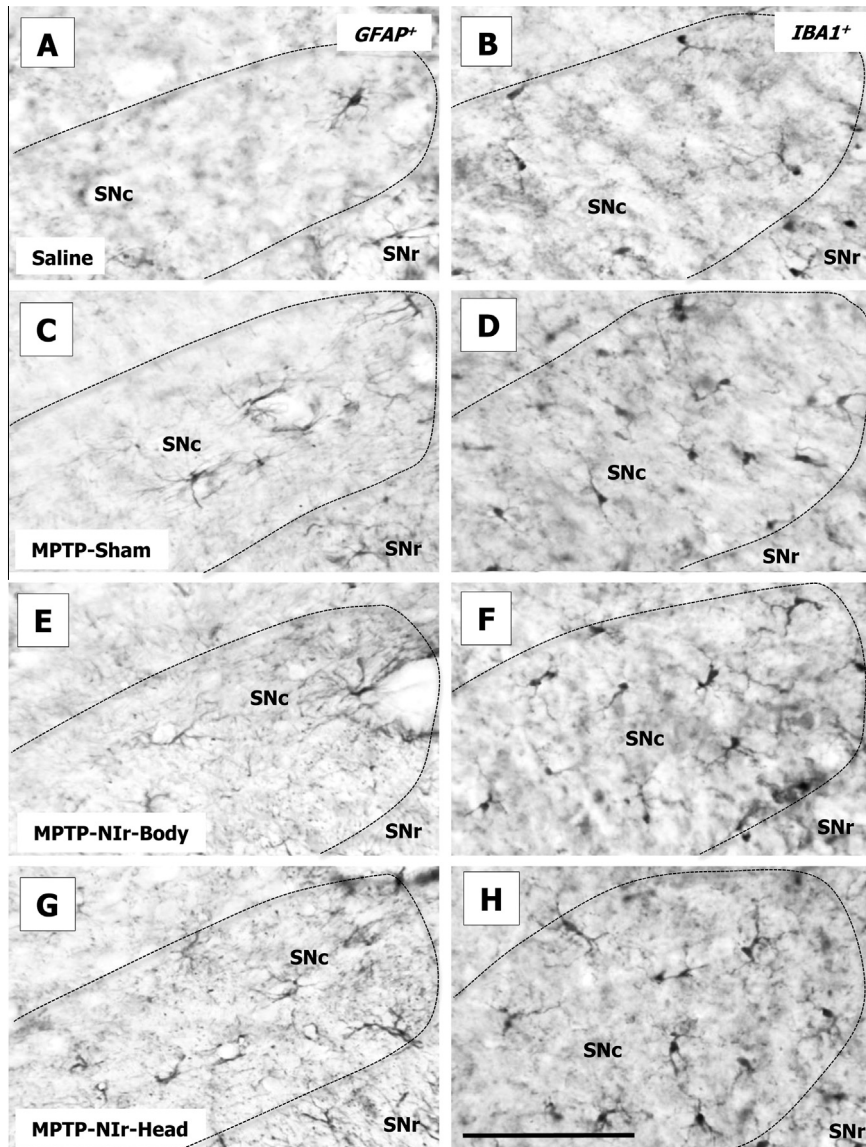


Fig. 5. Representative photomicrographs of glia in the SNc following 75-mg/kg MPTP with and without Nlr treatment. Immunolabeling of (A, C, E, and G) GFAP and (B, D, F, and H) IBA1 was used to assess the presence of astrocytes and microglia in the SNc of the (A, B) Saline, (C, D) MPTP-Sham, (E, F) MPTP-Nlr-Body and (G, H) MPTP-Nlr-Head experimental groups. All figures are of coronal sections; dorsal to top and lateral to right. Photomicrographs were taken from lateral regions of SNc, as indicated in Fig. 3C. Nuclear boundaries were determined by examining adjacent TH immunoreactive sections. Scale bar = 100 μ m.

more severe ischemic insults (Kharbanda et al., 2009; Jensen et al., 2011). Along similar lines, localized radiation therapy delivered to patients with metastatic cancer can occasionally result in regression of tumors distant from the site of irradiation. Termed the ‘abscopal effect’, this phenomenon is rare but widely reported, and is believed to be mediated by a systemic cytokine and/or immune response (Kaminski et al., 2005; Ludgate, 2012; Bramhall et al., 2014).

As most previous studies of the mechanisms that underpin the protective and regenerative effects of Nlr have focused on actions at the cellular level, there has been little investigation in the literature of exactly what circulating cells or molecules might mediate the systemic effect that we observe with remote Nlr. One of the prime

candidates appears to be bone marrow-derived stem cells, possibly mesenchymal stem cells. A series of studies by Tuby and colleagues has demonstrated that Nlr exposure increases the proliferation of c-kit-positive cells in the bone marrow and that, following myocardial infarction in rats, these cells are mobilized and recruited specifically to the site of damage where they contribute to a reduction in myocardial infarct size and ventricular dilatation (Tuby et al., 2007, 2009, 2011). This rescue is likely due to the capacity of these cells to release trophic factors that improve the function of damaged cells and aid in their survival (Tuby et al., 2006; Uccelli et al., 2011). Indeed, intravenous transplantation of exogenous mesenchymal stem cells has been shown to protect mouse dopaminergic neurons against MPTP toxicity

(Chao et al., 2009) and rat dopaminergic neurons against degeneration due to the proteasome inhibitor MG-132 (Park et al., 2008).

Alternative hypotheses to the actions of remote NIR include the modulation of immune cells, for example macrophages (Byrnes et al., 2005), and effects on inflammatory mediators, for example down-regulation of pro-inflammatory cytokines (interferon- γ , tumor necrosis factor- α) and up-regulation of anti-inflammatory cytokines (IL-4, IL-10) (Muili et al., 2012). Future studies will need to explore various lines of research in order to elaborate the exact mechanisms underlying the systemic response to NIR treatment.

It is important to note that the existence of a mechanism of indirect NIR-induced neuroprotection does not negate the well-studied direct cellular effects of NIR, which involve the stimulation of photoacceptors such as cytochrome oxidase and a consequent increase in respiratory chain activity and ATP production (Desmet et al., 2006; Huang et al., 2009; Rojas and Gonzalez-Lima, 2011; Chung et al., 2012). We suggest that these two mechanisms are not mutually exclusive, and that they may both contribute to the process of neuroprotection.

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