Vascular endothelial growth factor and platelet derived growth factor modulates the glial response to a cortical stab injury

Author
Norazit, Anwar, Nguyen, Maria, Dickson-Madondo, Charlotte, Tuxworth, Gervase, Goss, B, Mackay-Sim, Alan, Meedeniya, Adrian

Published
2011

Journal Title
Neuroscience

DOI
https://doi.org/10.1016/j.neuroscience.2011.06.035

Copyright Statement
Copyright 2011 International Brain Research Organization, published by Elsevier. This is the author-manuscript version of this paper. Reproduced in accordance with the copyright policy of the publisher. Please refer to the journal's website for access to the definitive, published version.

Downloaded from
http://hdl.handle.net/10072/42573
VEGF and PDGF modulates the glial response to a cortical stab injury

Anwar Norazit¹,², Maria Nga Nguyen¹, Charlotte G.M. Dickson¹, Gervase Tuxworth¹,³, Ben Goss⁴, Alan Mackay-Sim¹, Adrian C.B. Meedeniya¹,⁎

1. National Centre for Adult Stem Cell Research, Eskitis Institute for Cell and Molecular Therapies, Griffith University, Brisbane, Queensland, Australia
2. Department of Molecular Medicine, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia
3. School of Information and Communication Technology, Griffith University, Gold Coast, Queensland, Australia
4. Brisbane Spine Reference Centre, Institute of Health and Biomedical Innovation, Queensland University of Technology, Brisbane, Australia

⁎ Corresponding Author

Adrian C.B. Meedeniya

National Centre for Adult Stem Cell Research
Eskitis Institute for Cell and Molecular Therapies
Griffith University
Brisbane, Queensland 4111
Australia
ph: +61 7 3735 4417, Fax: +61 7 3735 4255
Email: A.Meedeniya@griffith.edu.au
Abstract

Traumatic injury to the brain initiates an increase in astrocyte and microglial infiltration as part of an inflammatory response to injury. Increased astrogliosis around the injury impedes regeneration of axons through the injury, while activated microglia release inflammatory mediators. The persistent inflammatory response can lead to local progressive cell death. Modulating the astrocyte and microglial response to traumatic injury therefore has potential therapeutic benefit in brain repair. We examine the modulatory effect of a single bolus of vascular endothelial growth factor (VEGF) and platelet derived growth factor (PDGF) in combination on astrocytes and microglia to acute cerebral injury. A combination of VEGF and PDGF (20 pg) was injected into the striatum of adult male Sprague-Dawley rats. The effects of treatment were assessed by quantitative immunofluorescence microscopy analyzing astrocytes and microglia across the stab injury over time. Treatment delayed the onset of astrogliosis in the centre and edge of the stab injury up to day 5; however, increased astrogliosis at areas remote to the stab injury up to day 5 was observed. A persistent astrocytic response was observed in the centre and edge of the stab injury up to day 60. Treatment altered microglia cell morphology and numbers across the stab injury, with a decrease in ramified microglia, but an increase in activated and phagocytic microglia up to day 5 after stab injury. The increased microglial response from 10 until day 60 was comprised of the ramified morphology. Thus, VEGF and PDGF applied at the same time as a stab injury to the brain initially delayed the inflammatory response up to day 5 but evoked a persistent astrogliosis and microglial response up to 60 days.

Keywords

vascular endothelial growth factor; platelet derived growth factor; astrogliosis; microglial response; brain injury.
Abbreviations

ANOVA, analysis of variance; PDGF, platelet derived growth factor; VEGF, vascular endothelial growth factor
1. Introduction

Traumatic injury to the brain initiates reactions in both astrocytes and microglia as part of an inflammatory response (Kreutzberg, 1996, Fawcett and Asher, 1999). Astrocytes, as an intermediate in neuron and blood vessel communication, have multiple roles in maintaining extracellular ion and fluid balance, clearing extracellular glutamate, regulating water transport, releasing or scavenging free radicals, and producing a wide range of pro- or anti-inflammatory cytokines and chemokines, growth factors, glucose and other energy metabolites (Katayama et al., 1990, Barres, 1991, Faden, 2002, Chen and Swanson, 2003, Kettenmann and Ransom, 2004). A stab injury to the cortex induces astrogliosis adjacent to the injury (Hampton et al., 2004) with the effect on astrocyte gene expression and hypertrophy dependent on the severity of the injury (Myer et al., 2006). This response re-establishes the blood brain barrier (Wang et al., 2004) and promotes survival of surrounding neurons (Sofroniew et al., 2001, Chen and Swanson, 2003). Although astrogliosis helps contain and restrict inflammation after brain injury (Myer et al., 2006), it impedes regeneration of axons through the injury site (Hampton et al., 2004) and can be detrimental by releasing pro-inflammatory cytokines or cytotoxic radicals (Kolker et al., 2001, Chen and Swanson, 2003).

A stab injury to the cortex induces an increased number of microglia at the injury site and their activation (Hampton et al., 2004, Batchelor et al., 2008). Microglia activation is evident in morphological changes in response to injury, classified as ramified, activated and phagocytic (Kreutzberg, 1996, Stence et al., 2001). Activated microglia produce pro-inflammatory mediators (reactive oxygen intermediates, nitric oxide, IFN-γ, TNF-α, and IL1) whose persistence leads to progressive cavitation at the site of injury (Gehrmann et al., 1995, Szczepanik et al., 1996, Fitch et al., 1999, Popovich et al., 2002, Hausmann, 2003).
Modulation of astrocyte and microglial response therefore remains a target for ameliorating the effects of traumatic brain injury (Vilhardt, 2005, Laird et al., 2008). This study applied two growth factors at the site of cortical stab injury, vascular endothelial growth factors (VEGF) and platelet-derived growth factor (PDGF), aimed at ameliorating the effects of focal brain injury. Previous work in our lab demonstrated that these factors, applied in combination acutely, significantly reduced astrogliosis and modulated the microglial response to prevent secondary degeneration following spinal cord injury (Lutton et al., 2011).

VEGF and PDGF have several known actions on astrocyte and microglia cell dynamics. Endogenous VEGF suppression induces astrocyte proliferation via autocrine signalling in the brain after injury (Krum and Khaibullina, 2003, Krum et al., 2008). In the brain, VEGF acts as a potent pro-inflammatory cytokine by up-regulating ICAM-1 and the chemokine MIP-1a (Croll et al., 2004) and inducing migration and proliferation of microglia in vitro (Forstreuter et al., 2002). Cultured astrocytes express PDGF and its receptors (Hutchins and Ard, 1993) and mediates glial differentiation (Noble et al., 1988, Raff et al., 1988, Richardson et al., 1988, Levine, 1989, Bogler et al., 1990, Wolswijk et al., 1991). Microglia also express PDGF (Nicholas et al., 2001), which interacts with the Iba-1 protein to enhance membrane ruffling, an important part of microglia motility and activation (Kanazawa et al., 2002). In this study VEGF and PDGF were delivered in combination during a stab injury to the rat cortex. The effects of these growth factors were assessed on the number and density of astrocytes and microglia at the injury site and surrounding it, for up to 60 days.
2. Experimental Procedures

2.1 Animal Surgery

Thirty adult male Sprague-Dawley rats (12 weeks; Animal Resource Centre, Western Australia) were randomised into 2 groups and housed in standard cages under controlled temperature and 12 hour light cycles, with food and water provided ad libitum. The treatment group received both 20 pg VEGF-165 (Biosource International) and 20 pg PDGF-BB (Biosource International) in Hanks Buffered Salt Solution containing 0.1% bovine serum albumin into the stab injury, whereas the control group received the same volume of Hanks Buffered Salt Solution containing 0.1% bovine serum albumin.

Stereotaxic surgery was conducted using protocol previously published (Norazit et al., 2010). Briefly, animals were anesthetized by isoflurane inhalation (Attane™, Bomac Australia). Using a stereotaxic frame (David Kopf Instruments, California), the cranium was exposed and using bregma as the reference point a burr hole was drilled at the co-ordinates: anterior-posterior = 1.2; lateral = 2.6; ventral = 5.0. Four µl of the solutions were infused at a rate of 1 µl/ minute using a Hamilton microsyringe (Hamilton Co., Reno). The burr hole was filled with an absorbable haemostat (Spongostan®, Johnson-Johnson Medical) and the incision sutured. The animals were provided with a prophylactic sub-cutaneous injection of the antibiotic oxytetracycline hydrochloride (Terramycin® 100, Pfizer Pty. Limited).

Animals (n=3/group/ time point) were euthanized 1, 2, 5, 10, and 60 days after surgery. The animals were euthanized with 1.5 ml of pentobarbitone sodium delivered intra-peritoneally (Lethabar® , Virbac, Australia). Animals were perfused transcardially with 0.5% sodium nitrite in 0.1M phosphate buffer saline followed by Zambonie’s fixative (2% paraformaldehyde and 0.2% picric acid in 0.1M phosphate buffered saline, pH 7.2). The brain was dissected clear and post-fixed in Zambonie’s fixative under vacuum. Animal experimentation was approved by the Griffith
University Animal Ethics Committee under guidelines of the National Health and Medical Research Council of Australia.

2.2 Immunohistochemistry

The immunohistochemistry protocol has been previously described (Nguyen et al., 2010, Norazit et al., 2010). Briefly, post-fixed brains were washed with 0.1M phosphate buffer saline and permeabilised in dimethyl sulphoxide. The tissue was placed in 30% sucrose dissolved in 0.05% sodium azide in 0.1M phosphate buffered saline and placed at 4°C overnight before being placed in a series of OCT (Sakura Tissue-Tek Oct Compound) solutions of increasing concentration (30 %, 50 % and 70 % in 30% sucrose dissolved in 0.05% sodium azide in 0.1M phosphate buffered saline). The brains were mounted in 100% OCT and sectioned on a cryostat to provide free floating sections of 40 µm thickness.

Sections were blocked with 10% normal donkey serum. Primary antibodies diluted using 10% normal donkey serum in 0.1M phosphate buffered saline, were applied to the sections and incubated overnight in a humidified chamber. Astocytes were identified using Rabbit anti- glial fibrillary acidic protein (DAKO; 1:1200) and Mouse anti- glial fibrillary acidic protein (Millipore; 1:1200). Microglia was identified using Rabbit anti- ionized calcium binding adaptor molecule 1 (WAKO; 1:2000), and axonal processes were identified using NF-200 (Sigma; 1:800).

Primary antibodies were removed and the sections washed with 0.1% Triton X-100 in 0.1M phosphate buffered saline. The secondary antibodies: Alexa Fluor® 594 Donkey anti Mouse IgG (Invitrogen 1:800); Alexa Fluor® 594 Donkey anti Rabbit IgG (Invitrogen 1:800); Alexa Fluor® 647 Donkey anti Mouse IgG (Invitrogen 1:40); Alexa Fluor® 647 Donkey anti Rabbit IgG (Invitrogen 1:40) diluted using 0.1% Triton X-100 in 0.1M phosphate buffered saline were applied to the sections. The secondary antibodies were removed and the sections washed with 0.1% Triton
X-100 in 0.1M phosphate buffered saline and mounted using Vectorshield DAPI (4’,6-diamidino-2-phenylindole·2HCl) mounting media (Vector Laboratories).

2.3 Image Acquisition

Images were acquired using a Zeiss Axioimager™ Z1, upright epi-fluorescence microscope, with Apotome, using 20X (dry) and 63X (oil immersion) Plan-Apochromatic objectives (numerical apertures of 0.75 and 1.40, respectively) (Carl Zeiss, Germany). MosaiX and standard wide field images were captured using an Axiocam Mrm camera with Axiovision™ image capture software (Carl Zeiss, Germany). Figures were compiled in Adobe Photoshop 11.1 and Adobe Illustrator 14 (Adobe Systems Incorporated). The digitized images were not manipulated apart from cropping, sizing and adjustment of contrast and brightness.

2.4 Densitometric measurements of astrogliosis (glial fibrillary acidic protein immunoreactivity)

Quantitative densitometric data was generated as a pixel density measure using custom MATLAB (The Mathworks) Software, using similar principles to those previously reported (Thiruchelvam et al., 2000, Norazit et al., 2010). Briefly, 3 optical sections (at 5μm, 20μm and 35μm) from a series of optical sections of the stab injury site were captured using a 20X objective. Glial fibrillary acidic protein and smooth muscle actin immunoreactivity was recorded in 0.014 mm² quadrants progressing stepwise from the centre of the stab injury to a distance of 0.42 mm. The sections were processed together and the exposure set to the brightest intensity to circumvent overexposure artefact. Background fluorescence was set by imaging a region devoid of glial fibrillary acidic protein. The images were converted to gray scale for analysing the staining intensity. Data is reported as a percentage of positive pixels (immunoflorescence) compared to background pixels (low or no signal).
2.5 Characterisation and quantification of microglia (ionized calcium binding adaptor molecule 1 immunoreactivity)

Cell number and morphology was recorded in 0.014 mm² quadrants progressing stepwise from the centre of the stab injury to a distance of 0.42 mm. Stereological principles were applied to the 3 dimensional data to allow accurate estimation of cell numbers although volume estimation and absolute cell numbers were not required (Peterson, 1999). Briefly, 2 tissue sections per animal were imaged with a 63X oil immersion objective, using the Apotome to capture a series of optical slices of the specimen. The data were analysed using 3D/4D image analysis software IMARIS x64 6.3.1 (Bitplane, Switzerland) to facilitate morphological characterization. Microglia were characterized using semi-quantitative methods to grade them as “resting”, “activated”, or “phagocytic” (Figure 1) (Kreutzberg, 1996, Stence et al., 2001).

2.6 Axonal outgrowth

Axonal outgrowth was recorded at the edge and at the centre of the stab injury using the 63X objective at Day 1, 2, 5, 10, 20, and 60 after stab injury. The effect of treatment induced astrocyte and microglia modulation on axonal outgrowth was assessed qualitatively as previously described (King et al., 2001).

2.7 Statistical Analysis

All results are presented as means and standard errors of the mean (SEM). Statistical significance was tested using two-way ANOVA and one-way ANOVA with a Newman-Keuls post-hoc analysis to prevent type 1 errors, using SPSS 17. Values of $p \leq 0.05$ were considered statistically significant.
3. Results

3.1 VEGF/PDGF treatment delayed and increased astrocytic density

An increase in astrogliosis is observed in the centre of the stab injury for up to 10 days in the untreated animals (Figure 2A & C). Astrogliosis then reduces to near normal levels by day 60 after stab injury. Treatment delayed the onset of astrogliosis in the centre of the stab injury for up to day 5 after stab injury, but evoked a persistent astrocytic response up to day 60. A two-way ANOVA indicated that the changes in astrogliosis in the centre of the stab injury is affected by time, \( (F(1,123) = 59.04, p \leq 0.0001) \) and by treatment \( (F(5,123) = 76.05, p \leq 0.0001) \). A significant interaction between time and treatment was also found \( (F(5,123) = 47.03, p \leq 0.0001) \) (Figure 2C).

VEGF and PDGF treatment altered the spatial density of astrocytes across the stab injury (Figure 3). Treatment delayed astrogliosis at the centre and edge of the stab injury but increased astrogliosis at areas remote to the stab injury up to day 5 following injury \( (p \leq 0.01, p \leq 0.05) \). At day 10 after stab injury, there was no apparent difference in the astrocytic response across the stab injury between treatment and control. At day 20 after stab injury, an increased astrogliosis was seen at the centre and edge of the stab injury in response to treatment. At day 60 after stab injury, astrogliosis was present at the centre and edge of the stab injury in response to treatment \( (p \leq 0.01, p \leq 0.05) \); however, a reduction in astrocytic density in comparison to day 20 was apparent (Figure 3). The glial density in the control injury had reached basal levels by day 60.

3.2 VEGF/PDGF treatment delayed and increased the numbers of microglia

An increase in total microglia numbers is observed in the centre of the stab injury for up to 5 days (Figure 2B &D) in the untreated animals. The number of total microglia then returns to near basal levels by day 60 after stab injury. Treatment increased total microglia numbers up to day 2 after stab injury before a further dramatic increase at day 5. Total microglia continued to increase up to day 10 after stab injury, before beginning to decline and reaching near basal levels at day 60. A
Two-way ANOVA indicated that the changes in total microglia infiltration in the centre of the stab injury is affected by time ($F(1,30) = 140.11, p \leq 0.0001$) and by treatment ($F(5,30) = 70.93, p \leq 0.0001$). Finally, a significant interaction between time and treatment was found ($F(5,123) = 43.66, p \leq 0.0001$) (Figure 2D).

VEGF and PDGF treatment altered microglia cell morphology and numbers across the stab injury site (Figure 4). Treatment delayed the infiltration of ramified microglia ($p \leq 0.01, p \leq 0.05$), with no apparent increase in activated microglia across the stab injury at day 1. No phagocytic microglia was present in either control or treatment stab injuries at this time. At day 2 after stab injury, treatment caused a similar distribution pattern of ramified microglia with the control stab injury with an increase in activated microglia at the edge and at areas adjacent to the stab injury ($p \leq 0.01$). An increase in phagocytic microglia is present in the centre of the stab injury following treatment, with no increase seen in the control stab injury. Treatment continued to delay ramified microglia infiltration at the stab injury ($p \leq 0.05$) up to day 5; however an increase in ramified microglia infiltration at the edge and areas remote to the stab injury ($p \leq 0.05$) was apparent. Treatment substantially increased activated and phagocytic microglia ($p \leq 0.01$) at day 5 after stab injury. At day 10 after stab injury, no activated or phagocytic microglia was present in both treatment and control stab injuries. Treatment evoked an increase in ramified microglia at centre, edge and areas adjacent to the stab injury ($p \leq 0.01, p \leq 0.05$). At day 20 after stab injury, treatment increased the ramified microglia at centre, edge and areas remote to the stab injury ($p \leq 0.01, p \leq 0.05$); however, a reduction in ramified microglia in comparison to day 10, was apparent. By day 60 after stab injury, treatment showed an increase in ramified microglia at the centre of the stab injury ($p \leq 0.01$); however, a reduction in ramified microglia in comparison to day 20 was apparent, with ramified microglia at the edge and areas remote to the stab injury returning to basal levels.
3.3 VEGF/PDGF treatment stimulates axonal growth

The stab injury with or without treatment (n=3/group) resulted in clear tissue disruption at day 1 and 2 after stab injury. Large “bulb” like swellings and discrete ring shaped NF-200 accumulations was apparent at the edge of the stab injury (Figure 5A & B). At day 5 after stab injury, small sprout like protubences emerging from the “bulb” like swellings was observed in both control and treatment groups (n=3/group) (Figure 5C). Notably, in all treated animals (n=3), an increase in NF-200 positive processes associated with microglia cells were recorded at the edge of the injury (Figure 6). A relatively low level of NF-200 positive processes associating with microglia cells was observed in the control animals (n=3). At day 10, 20, and 60 after treatment (n=3/group/timeline), an increase in NF-200 positive processes associated with microglia at the edge of the injury were observed compared to their time point matched control. However, in all control and treated animals (n=30), no NF-200 positive processes crossed the stab injury site.
4. Discussion

This study demonstrates that treatment with VEGF and PDGF significantly alters the dynamics of astrocytes and microglia response to a stab wound injury of the cortex. Growth factor treatment delayed the injury-induced increase in numbers of astrocytes and microglia at the injury site and altered the spatial distribution of astrocytes and microglia around it. Growth factor treatment appeared to stimulate axon sprouting into the injury site.

The effects of VEGF and PDGF is dose-dependent. At low doses, as used in the present study, VEGF can be neuroprotective \textit{in vitro} and neurotrophic \textit{in vivo} (Pietz et al., 1996, Pitzer et al., 2003) but detrimental on injection into the brain at high doses (Shih et al., 2004, Yasuhara et al., 2005). Similarly, chronic treatment with VEGF increased astrogliosis at day 3 (Krum et al., 2002), rather than delayed astrogliosis as seen with a single bolus dose in combination with PDGF in the present study. Some of the effects of VEGF and PDGF treatment may arise indirectly. For example, axon sprouting may have resulted from the increase in activated microglia, which is a reported inducer of dopaminergic axon sprouting after cortical injury (Batchelor et al., 1999) possibly via the expression of BDNF and GDNF (Batchelor et al., 1999, Dougherty et al., 2000).

The time-dependent effects of VEGF and PDGF treatment on astrocytes and microglia suggest that the treatment has modulated the inflammatory process, perhaps even accelerating its resolution. Treatment delayed the injury-induced increase in the numbers of astrocytes and microglia at the injury site and then increased these numbers above those achieved sooner in the control animals. Treatment additionally altered the dynamics of microglia of different morphologies. For example, 5 days after treatment there was an increase in phagocytic microglia, which are thought to originate from the activated microglia. This transition is indicated by the concomitant reduction in activated microglia at day 5, even with increased numbers at day 2 after treatment. Phagocytic microglia digest and eliminate the cellular debris resulting from the injury and the cytotoxic effects of
activated microglia (Vilhardt, 2005), thus this altered dynamic indicates an accelerated transition to anti-inflammatory microglia with more rapid resolution of inflammation.

Ten days after treatment only ramified microglia were apparent in the stab injury site. Ramified microglia returned to near basal levels in the time point matched controls. Activated microglia has the ability to return to their resting ramified state (Hailer et al., 1996, Hailer et al., 1997), thus the absence of activated and phagocytic microglia may reflect their return to a ramified state. At day 10 after treatment, there was no apparent difference in the astrocytic response across the stab injury between treatment and control. The increase in astrocytes may indirectly help support the damaged/regrowing neurons via neurotrophic factor support (BDNF, GDNF, NGF) (Schwartz and Nishiyama, 1994, Dougherty et al., 2000, Mizuta et al., 2001). NF-200 positive processes were still apparent at the edge of the injury in both control and treated animals; however, none of these processes were observed crossing the stab injury site. It has been demonstrated that neurofilament expressing axons cross a stab injury in the neocortex of rats at day 7 after injury (King et al., 2001) suggesting that the treatment-induced increase in astrogliosis may impede axonal regrowth. The increase in astrogliosis at the injury site may be due to the clearing of cellular debris by the phagocytic microglia, thus leaving an area void of tissue. The rapid increase in astrogliosis and ramified microglia in the treated animals compared to the controls suggest a decrease in the modulatory effect of VEGF and PDGF. This proposes the prospect of a prolonged modulatory effect by continues exposure of VEGF and PDGF.

Astrocytes and ramified microglia returned to near basal levels in the control group at day 20 and 60 after stab injury. The treatment-induced increase in astrogliosis and ramified microglia was higher at day 20 compared to day 60. As an injury resolves, a smaller percentage of ramified microglia remain motile (Rezaie et al., 2002, Nimmerjahn et al., 2005), moving away from the
injury site. Contact between microglia is also capable of inducing motility of neighbouring microglia (Petersen and Dailey, 2004).

Whilst neither control nor treatment groups showed NF-200 positive processes crossing the injury site, the experiment shows that a combined dose of VEGF and PDGF at the time of a stab injury to the brain delays astrogliosis and ramified microglia infiltration, while increasing activated and phagocytic microglia. Providing neurotrophic support may facilitate axonal growth by increasing microglia activation whilst delaying astrogliosis thus extend the window of opportunity for axons to cross the injury site before the establishment of the astrocyte barrier.

Acknowledgements

We would like to thank Brenton Cavanagh, Joseph Kan, Will Young, and Cameron Lutton for their technical support. This work was funded by a grant from the National Health and Medical Research Council and the Australian Department of Health and Ageing to A.M.S.
References


Figure 1: Microglia morphology. (a) Ramified microglia with a cell body between 30-60 µm² and extensive fine processes (b) Activated microglia with a cell body between 60-150 µm² with a few thick processes (c) Phagocytic microglia with a cell body greater than 150 µm² and no processes. Microglia was stained using ionized calcium binding adaptor molecule 1 (IBA-1; red). Nuclei are counter stained with the nuclear marker DAPI (blue). (Scale bar = 10 µm).
Figure 2: VEGF and PDGF altered the dynamics of astrocytes and total microglia after stab injury. A & C: Treatment delayed astrogliosis, defined with glial fibrillary acidic protein (GFAP; red) at the centre of the stab injury (box; white) up to day 5 in comparison to the control. A distinct increase in astrogliosis was observed from day 20 after treatment. A significant interaction between time and treatment was found ($F(5,123) = 47.03$, $p \leq 0.0001$) using two-way ANOVA. B & D:
Treatment did not have any apparent effect on total microglia stained using ionized calcium binding adaptor molecule 1 (IBA-1; green) in the centre of the stab injury up to day 2 in comparison to the control. Treatment increased total microglia on day 10 and 20 after treatment before returning to near basal levels on day 60 after stab injury. A significant interaction between time and treatment was found ($F(5,123) = 43.66, p\leq0.0001$) using two-way ANOVA. Nuclei are counter stained with the nuclear marker DAPI (blue). (Scale bar = 100 µm). (All data are expressed as mean values ± SEM).
Figure 3: VEGF and PDGF alter the spatial densities of astrocytes across the stab injury.

Treatment delayed astrogliosis at the centre and edge of the stab injury but increased astrogliosis at areas adjacent to the stab injury site up to day 5 after stab injury ($p \leq 0.01$, $p \leq 0.05$) At day 10 after stab injury, treatment showed no statistically significant difference in astrogliosis across the stab injury; however treatment increased astrogliosis at the centre and edge of the stab injury on day 20 and day 60 ($p \leq 0.01$, $p \leq 0.05$). All data are expressed as mean values ± SEM and analysed using one-way ANOVA with a Newman-Keuls post-hoc analysis.
Figure 4: VEGF and PDGF alter spatial numbers of microglia across the stab injury.

Treatment delayed ramified microglia infiltration in the stab injury up to day 5 ($p \leq 0.01$, $p \leq 0.05$).

Treatment only increased infiltration of ramified microglia at areas adjacent to the stab injury on day 5 after stab injury ($p \leq 0.01$). An increase in activated and phagocytic microglia is present in the
centre and edges of the stab injury following treatment up to day 5 after stab injury ($p \leq 0.01$).

Treatment evoked an increase in ramified microglia at centre, edge and areas adjacent to the stab injury at day 10 and 20 after stab injury ($p \leq 0.01$, $p \leq 0.05$); however, a reduction in ramified microglia on day 20 after stab injury in comparison to day 10 after stab injury was apparent. At day 60 after stab injury, treatment continued to show an increase in ramified microglia at centre of the stab injury ($p \leq 0.01$); however, a reduction in ramified microglia in comparison to day 20 was apparent, with ramified microglia at the edge and areas remote to the stab injury returning to basal levels. All data are expressed as mean values ± SEM and analysed using one-way ANOVA with a Newman-Keuls post-hoc analysis.

Figure 5: Axonal response after a stab injury.

a) Large “bulb” like swellings (hollow arrows) associated with “reactive” abnormal axons. b) Discrete ring shaped NF-200-immunoreactive structures (filled arrows) associated with “reactive” abnormal axons. c) Small sprout like protubences (hollow arrow) emerging from the “bulb” like swellings associated with axonal regrowth. (scale bar = 50 µm)
Figure 6: VEGF and PDGF stimulate axonal growth.

a) Treatment resulted in an apparent increase in neurofilament (NF-200; red) processes associated with microglia (IBA-1; green) at the edge of the stab injury (outlined in white) at day 5 after treatment. b) Lack of neurofilament (NF-200; red) processes associated with microglia (IBA-1; green) at the edge of the stab injury was apparent at day 5 in the control cohort. (scale bar = 50 µm)