Stimulating Circle of Willis Nerve Fibers Preserves the Diffusion-Perfusion Mismatch in Experimental Stroke

Nils Henninger, MD; Marc Fisher, MD

Background and Purpose—Stimulation of the nerves traversing the ethmoidal foramen (including postsynaptic, parasympathetic projections from the sphenopalatine ganglion [SPG], henceforth referred to as “SPG-stimulation”) has been shown to elevate cerebral blood flow (CBF) and to be neuroprotective after permanent, middle cerebral artery occlusion (pMCAO).

Methods—Employing diffusion (DWI)- and perfusion (PWI) weighted MRI, the effect of SPG-stimulation (started at 60 minutes post-MCAO) on the spatiotemporal evolution of ischemia during and after pMCAO was investigated. In an additional experiment, regional CBF changes were investigated in the nonischemic brain.

Results—In the nonischemic brain, SPG stimulation significantly elevated CBF predominantly within areas supplied by the anterior cerebral artery (by 0.64 mL/g/min relative to baseline). In the ischemic brain, CBF only marginally increased within the penumbra and core (by up to 0.08 and 0.15 mL/g/min relative to prestimulation, respectively). However, the threshold-derived CBF lesion volume did not change significantly. Penumbral apparent diffusion coefficient (ADC)-values improved to almost baseline values and the threshold derived ADC/CBF-mismatch was preserved up to 180 minutes after MCAO. TTC-derived lesion volumes were significantly smaller in stimulated versus nonstimulated animals (120.4 ± 74.1 mm³ versus 239.3 ± 68.5 mm³, respectively).

Conclusion—This study demonstrates that unilateral SPG-stimulation increases CBF bilaterally within the normal brain, acutely preserves the CBF/ADC mismatch largely independent of altering cerebral blood flow, and reduces infarct size in the rat permanent suture model. (Stroke. 2007;38:2779-2786.)

Key Words: diffusion weighted imaging ■ focal cerebral ischemia ■ perfusion weighted imaging ■ pterygopalatine ganglion ■ suture model

It is well established that hypoperfusion below a critical threshold as a consequence of embolic/thrombotic compromise of cerebral blood flow (CBF) is the key event leading to ischemic cell death. The obvious therapeutic response to such a reduction of blood supply is to reestablish flow by pharmacologic or mechanical means as quickly as possible to reduce infarct size and improve functional/neurologic outcome.1 To date, the only proven thrombolytic therapy for stroke is treatment with recombinant tissue plasminogen activator (rtPA). However, thrombolysis with intravenous rtPA has many limitations, including a short treatment time window, recanalization rates of only approximately 50%, and a substantial risk of symptomatic hemorrhagic transformation.2 Hence, there remains substantial interest in discovering novel therapeutics for use in acute cerebral ischemia.

In the rat, parasympathetic nerve fibers derived from the sphenopalatine (or pterygopalatine) ganglion (SPG) traverse the intracranial space through the ethmoidal foramen, innervating cerebral arteries.3,4 It was shown that stimulation of these postsynaptic, parasympathetic SPG projections as well as the ethmoidal branch of the nasociliary nerve (a trigeminal branch) near the ethmoidal foramen increases CBF, presumably via vasodilation of cerebral arteries secondary to the release of nitric oxide (NO) and vasoactive intestinal peptide (VIP).5,7 The administration of NO to animals after stroke increases CBF in the ischemic territory and reduces tissue damage.8 Furthermore, it was recently demonstrated that stimulation of the aforementioned traversing nerves (subsequently referred to as “SPG-stimulation” because of the prevailing parasympathetic SPG-projections within this nerve bundle) in the rat increased cortical blood flow, reduced final infarct size, and improved neurological outcome after permanent middle cerebral artery occlusion (pMCAO).9

The prior study focused on final lesion size and regional blood flow as assessed by classic histological techniques and laser doppler flowmetry. The spatiotemporal dynamics of the ischemic penumbra was not evaluated. Diffusion- (DWI) and perfusion-weighted (PWI) imaging provides a powerful methodology to identify ischemic tissue at risk for infarction by assessing the DWI/PWI mismatch.10 The purpose of this study was to compare the spatiotemporal lesion evolution during the acute phase of pMCAO before as well as after SPG...
stimulation. Serial determination of quantitative CBF and apparent diffusion coefficient (ADC) maps were performed up to 3 hours after pMCAO and correlated with 24-hour 2,3,5-triphenyltetrazolium chloride (TTC)-derived infarct volumes.

Materials and Methods

Animal Preparation

All procedures used in this study were performed in accordance with our institutional guidelines and all experiments were performed in a blinded, randomized manner. To alleviate pain, animals received 0.05 mg/kg subcutaneous buprenorphine immediately as well as 6 hours after the end of anesthesia. Male Sprague Dawley rats (n=21, Taconic Farms, New York) weighing 290±15 g were anesthetized with isoflurane (5% for induction, 2% for surgery, 1.5% for maintenance) in room air. PE-50 polyethylene tubing was inserted into the left femoral artery for continuous monitoring of mean arterial blood pressure (MABP) and for obtaining blood samples to measure pH, PaO2, P aCO2, and plasma glucose at baseline, 30, 60, and 180 minutes after pMCAO. Body temperature was maintained at 37.0±0.5°C with a thermostatically controlled water pad. Permanent focal cerebral ischemia was produced by intraluminal suture occlusion of the right middle cerebral artery using 4-0 silicon-coated monofilament sutures as previously described. Neurologic evaluation was performed at 24 hours as previously described.

Study Design

The study consisted of 2 different experiments:

Experiment 1 aimed to investigate the effect of SPG-stimulation on regional CBF of animals not subjected to pMCAO. Rats were randomized 2:1 to one of the following groups: After a baseline scan, SPG-stimulation was performed at 0 and 15 minutes, respectively (SPG, n=6). Control animals were operated and had the stimulating electrode implanted but were not stimulated (control, n=3). All animals were euthanized immediately after the end of the last imaging session.

Experiment 2 aimed to investigate the effect of SPG-stimulation on regional CBF after induction of pMCAO. Rats were randomized to 1 of the following groups: After a baseline scan, SPG-stimulation commenced at 60 minutes post-MCAO and was repeated every 15 minutes until after 3 hours after MCAO (SPG-MCAO, n=6). Control animals were subjected to pMCAO and had the stimulation electrode attached but were not stimulated (Control-MCAO, n=6). At 24 hours after MCAO animals were euthanized by an overdose of intraperitoneal injection of pentobarbital (200 mg/kg) followed by decapitation. Brains were removed and sectioned coronally into 7 1.5-mm-thick slices corresponding to the MR slices and stained with 2,3,5-triphenyltetrazolium chloride (TTC) for post mortem infarct volume calculation with edema correction. Absolute and relative swelling of the right (ischemic) versus left hemisphere was assessed. A corrected infarct volume was calculated by the following formula: corrected infarct volume = left hemisphere volume – (right hemisphere volume – infarct volume).

Electrical Stimulation

The head of the animal was secured in an MR-compatible stereotactic frame and a fine, bipolar, hook-shaped stimulating electrode was put under the nerve bundle that extended from the sphenopalatine ganglion (SPG) to the ethmoidal foramen as previously described. The electrode was then immersed in mineral oil, the wound closed, and the electrode fastened to the stereotactic frame. Such secured animals were placed into the MR-scanner without risk of displacement of the stimulating electrode. The electrode was connected to an electronic stimulator (Neuropath, BrainsGate LTD). Four sets of 60-second stimulations separated by 12-second intervals were administered at 10 Hz (pulse width=0.2 ms) with an intensity of 1.9 to 2.2 mA corresponding to a peak level of 60 mV. The stimulation paradigm was optimized during a previously published study, yielding robust neuroprotection without producing nerve injury, changes in animal physiology or disruption of the blood–brain barrier (BBB), that only occurred with higher (>10 mA) stimulation intensities and longer pulse widths (1 ms, data not shown).

MRI Measurements

MRI experiments were performed on a 4.7 T/40 cm horizontal magnet equipped with a Biospec Bruker console (Billerica), and a 20 G/cm gradient insert (ID=12 cm, 120-μs rise time). A surface coil (ID=2.3 cm) was used for brain imaging and an actively decoupled neck coil for perfusion labeling. Animals were imaged at baseline, 0, 5, 10, 15, 20, 25, 30, 60, 90, 120, 150, and 180 minutes (experiment 1) and at 30, 60, 90, 120, 150, and 180 minutes after MCAO (experiment 2), respectively. The ADC of water and CBF were recorded. Three ADC maps were separately acquired with diffusion-sensitive gradients applied along the x, y, and z direction. Single shot, echo-planar images (EPI) were acquired over 2.5 minutes with matrix=64x64, spectral width=200 kHz, TR=2 s (90° flip-angle), TE=37.5 ms, b=8 and 1400 s/mm², Δ=24 ms, δ=4.75 ms, FOV=2.56x2.56 cm, 7 1.5-mm slices, and 16 averages. CBF measurements were made using the continuous arterial spin-labeling (CASL) technique with single-shot, gradient-echo, EPI acquisition. When scheduled at the same time, CBF measurements were made during SPG-stimulation. Note that in MCAO-animals, the 60-minute DWI scan started immediately after the first SPG-stimulation (ie, at 65 minutes post-MCAO). Paired images were acquired alternately—one with arterial spin labeling and the other without. MR parameters were as follows: matrix=64x64, TR=2 s (90° flip-angle), TE=13.5 ms, FOV=2.56x2.56 cm, 7 1.5-mm slices, and 60 pairs of images.

Region-of-Interest Analysis

Only the 5 most anterior slices (ie, brain tissue from approximately Bregma +4 mm to −2 mm) were used for region-of-interest (ROI) analysis because the SPG is the source of parasympathetic innervation to most of the anterior part of the cerebral vasculature. Additionally, we did not observe overt CBF-changes within the parieto-occipital cortex (supplied through the posterior parts of the circle of Willis) during or after SPG-stimulation (data not shown). In experiment 1, hemispheric CBF was calculated to investigate the overall effect of SPG-stimulation on cerebral blood flow. In addition, relative CBF changes were calculated for the individual MR slices (with slice 1 being most anterior). Lastly, three ROIs (each 4x4 pixels) were manually defined in both experiments as follows: (ROI1) Caudate putamen (ischemic core in experiment 2), (ROI2) middle cerebral artery supplied cerebral cortex (ischemic penumbra in experiment 2), and (ROI3) anterior cerebral artery supplied cortex (non-ischemic tissue in experiment 2). Care was taken to ensure that ROIs were placed in the same location within the ipsi- and contralateral hemispheres, across groups, images, and time points. Further, ROIs were drawn conservatively avoiding the brain-skull interface as well as the ventricles.

Calculation of In Vivo Lesion Size

Images were analyzed using STIMULATE (University of Minnesota) and QuickVol II (http://www.quickvol.com/). Quantitative average ADC and CBF maps and their corresponding threshold-derived lesion volumes were calculated as described elsewhere. Briefly, viability thresholds were used to identify all pixels with abnormal ADC and CBF characteristics on each of the 7 imaged slices at each time point. The corresponding ADC and CBF lesion volumes were then calculated by summing the abnormal area and multiplying by the slice thickness. The viability thresholds used were 0.53x10⁻³ mm²/s for ADC and 0.3 mL/g/min for CBF, as previously validated.

Statistical Analysis

Data are presented as mean±SD unless otherwise stated. Statistical comparisons (Sigma-Stat 3.1, SPSS) were performed using repeated
measures analysis of variance (RM-ANOVA) with post hoc Holm-Sidak or Dunn test for multiple comparisons, 2-way RM-ANOVA, and 2-tailed paired or unpaired Student t test, where appropriate. P/H110210.05 was considered significant.

Results

Physiological Parameters and Neurological Score

No animals died and basal physiological parameters did not differ significantly among groups or experiments at any time point (Table), and there was no significant difference in weight among animals of both groups (data not shown). No significant changes in MABP were observed during and after SPG-stimulation relative to values obtained at baseline as well as relative to controls (Table; Figure 1B), indicating sufficient depth of anesthesia to prevent nonspecific CBF-changes attributable to stimulation induced arousal. The 24-hour neurological scores (mean±SD min, max, range)

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Physiological parameters obtained from experiment 2 (pMCAO). There was no significant difference in glucose, pH, Pco2, and Po2 levels within and between pMCAO-groups. Furthermore, continuous measurement of MABP as well as temperature did not reveal any changes within and between pMCAO-groups (for clarity, only data from selected time points are shown).

Figure 1. A, Four representative CBF maps (Bregma +4.0 to −0.5 mm) of an SPG-stimulated rat. B, Blood pressure recording from the same animal as shown in A over the 180 minutes imaging period. C, Five representative MR and histological images of an SPG-stimulated pMCAO rat at selected time points showing ADC and CBF maps as well as TTC staining. Threshold-derived ADC lesion values as well as CBF values are indicated according to the color scales. Black arrows in C indicate ADC/CBF mismatch, white arrows indicate persistence of a large threshold-derived CBF lesion at 180 minutes, and white arrowheads indicate infarct reduction at 24 hours as assessed by TTC staining.
were improved ($P=0.041$) in the SPG group (2.5±0.8, 2, 4, 2) relative to controls (3.7±0.8, 3, 5, 2).

**Experiment 1: Quantitative Apparent Diffusion Coefficient and Cerebral Blood Flow Values Within ROIs**

One stimulated animal did not show any CBF changes throughout the experiment and was subsequently excluded from the data analysis as damage to the nerve bundle was observed. Stimulation induced a significant increase (by $\approx$25%, range 14% to 76%) in hemispheric CBF relative to baseline. Though animals received only unilateral stimulation, CBF increased similarly in both hemispheres without significant side-by-side differences (data not shown). However, the observed CBF-increases were only significant during stimulation with almost immediate renormalization to baseline values after stimulation. Calculation of the absolute CBF-changes between baseline and subsequently measured CBF maps suggested greatest SPG-stimulation induced changes within anterior parts of the brain (Figure 1A). Relative to baseline values, the first stimulation induced significant CBF-elevations only in slice 1 (1.4±0.5 [range 0.9 to 2.2] mL/g/min). With the second stimulation, significant CBF-elevations were observed in slice 1 (1.4±0.4 [range 0.90 to 2.0] mL/g/min), slice 2 (1.4±0.2 [range 1.2 to 1.8] mL/g/min), and slice 3 (1.5±0.3 [range 1.1 to 2.0] mL/g/min), respectively. These findings suggested that there was a cumulative effect on CBF with subsequent stimulations with a greater contribution from the anterior cerebral artery (ACA) than from the middle cerebral artery (MCA) territory. Indeed, calculation of relative CBF-changes (data not shown) clearly showed a more pronounced effect of SPG-stimulation in the anterior (mostly ACA-supplied) versus mid-posterior (mostly MCA-supplied) parts of the brain. Furthermore, in contrast to ROIs 1&2 (predominantly MCA-supplied, increases by $\approx$16 and 31%, respectively), only ROI3 (ACA-cortex, increase by $\approx$56% from baseline) showed significantly higher CBF values relative to control animals after the second SPG-stimulation (Figure 2).

**Experiment 2: Temporal Evolution of Apparent Diffusion Coefficient and Cerebral Blood Flow-Derived Lesion Volumes and Correlation With TTC-Derived Infarct Volumes**

Figure 1C shows representative MR images of a pMCAO rat at various time points before and after SPG-stimulation, and Figure 3 shows the spatiotemporal evolution of CBF and ADC lesion volumes as well as 24-hour TTC-derived infarct sizes. The CBF lesion volume did not differ between groups and remained relatively constant over time. In controls, no significant difference between ADC and CBF lesion volumes existed by 60 minutes and the TTC-derived lesion volume almost matched the 3-hour CBF/ADC lesion volumes ($P>0.05$). Furthermore, there were no significant differences in absolute (49.2±35.7 mm$^3$ versus 70.3±32.2 mm$^3$, $P=0.307$) and relative (8.4±6.4% versus 10.8±5.3%, $P=0.485$) hemispheric swelling between stimulated and nonstimulated MCAO-groups at 24 hours, as assessed on TTC-stained brain sections. In contrast, SPG-stimulation significantly preserved the DWI/PWI-mismatch during treatment and significantly reduced the final TTC-derived lesion volume (120.4±74.1 mm$^3$) relative to nonstimulated controls (239.3±68.5 mm$^3$). After SPG stimulation, the CBF-derived lesion volumes in stimulated animals were approximately 10% smaller (nonsignificant, $P>0.05$) than in nonstimulated controls.

**Quantitative Cerebral Blood Flow Values Within ROIs**

Figure 4A and 4C shows region specific CBF values within investigated ROIs of both pMCAO-groups. There was a statistically significant increase in core (ROI1) and mismatch (ROI2) CBF after SPG stimulation; however, these changes were only marginal ($\approx$0.1 mL/g/min) and elevated regional CBF only close to or slightly above the viability threshold. In
contrast to nonstroke animals, CBF did not change significantly within the ipsilesional (range 0.84±0.24 to 0.95±0.13 mL/g/min) and contralesional (range 0.89±0.36 to 1.05±0.15 mL/g/min) ACA-cortex (ROI3). Furthermore, CBF did not significantly differ within or between contralesional ROIs over the course of the experiment (data not shown).

Quantitative Apparent Diffusion Coefficient Values Within ROIs

Figure 4B and 4D shows region-specific ADC values within investigated ROIs of both pMCAO-groups. There was a significant improvement of mismatch (ROI2) but not core (ROI1) ADC values above the threshold for infarction after SPG-stimulation (P<0.05 vs nonstimulated controls). ADC values did not change significantly within the ipsilesional and contralesional ACA-cortex (ROI3). Furthermore, ADC values did not significantly differ within or between contralesional ROIs over the course of the experiment (data not shown).

Discussion

Several studies have shown that stimulation of the SPG in anesthetized animals induces vasodilation of cerebral arteries and elevations in CBF as assessed by laser Doppler flow studies,3,9 mass spectrometry,5 or angiography.6,7 Based on these findings it was hypothesized that electrical stimulation of the SPG may improve hemispheric CBF during focal cerebral ischemia and attenuate final infarct size.9 Indeed, Yarnitsky et al9 demonstrated a moderate improvement of cortical CBF during permanent MCAO as well as decreased 27-hour infarct volumes. However, this study did not systematically assess the acute evolution of the ischemic lesion. Using DWI and PWI, we investigated the effect of SPG-stimulation on the temporal evolution of CBF changes in the nonischemic as well as ischemic rat brain. In addition, we assessed the spatiotemporal evolution of ischemia during and after pMCAO as well as ascertained regional changes of quantitative ADC and CBF values within selected ROIs in stimulated versus nonstimulated MCAO rats.

The major results of this study were that: (1) SPG-stimulation significantly elevated CBF predominantly within the nonischemic brain supplied by the anterior cerebral artery; (2) the observed CBF-alterations were brief in nature (peristimulational) and less pronounced compared with previously reported results obtained by laser Doppler flow studies;3 (3) in the ischemic brain, CBF only marginally increased and did not contribute to a significant decrease in threshold derived CBF lesion volume; (4) intriguingly, stimulation improved penumbral ADC-values to almost baseline values preserving the DWI/PWI-mismatch; (5) lastly, final TTC-derived lesion volumes were significantly smaller in SPG-stimulated versus nonstimulated animals.

Electrical stimulation of the nasociliary nerve and SPG in the rat has been shown to transiently increase CBF together with MABP without affecting heart rate, and it was hypothesized that the rapid increase of MABP after stimulation could contribute to CBF elevation.5 Conversely, this as well as other studies did not observe significant changes in MABP during or after SPG stimulation,4,7 possibly because of interstudy differences in stimulation parameters and anesthesia. In this respect it is of note that isoflurane is a potent cerebrovasodilator leading to a global increase in CBF. This could have resulted in mitigation of MABP-associated CBF changes and such a “ceiling effect” may also explain the less pronounced stimulation-induced CBF changes in the ipsilateral parietal cortex of this study (~30%) relative to previously reported values ranging from ~40% to 100%.3,5 Indeed, whereas baseline CBF was higher in isoflurane versus alpha-chloralose anesthetized animals, CBF-changes after forepaw stimulation were much less than under alpha-chloralose, indicating that isoflurane reduces relative CBF-changes as a result of its vasodilating properties.17

Unexpectedly, we observed similar CBF-changes bilaterally as opposed to the previously reported predominantly

Figure 3. Spatiotemporal evolution of threshold-derived ADC and CBF lesion volumes of stimulated and nonstimulated pMCAO rats. *P<0.05 for significant CBF/ADC mismatch volume (paired t test); ‡P<0.05 for between-group differences of CBF or ADC lesion volumes (Student t test).
isphemispheric CBF-elevations.\textsuperscript{3–5} Fibers from each sphenopalatine ganglion innervate the contralateral hemisphere in the rat,\textsuperscript{18} which partly explains the albeit small, yet significant contralateral increases in CBF with SPG-stimulation.\textsuperscript{3,5} In addition, both anterior cerebral arteries in the rat fuse to form the azygos anterior cerebral artery, therefore, unilateral vaso-
dilation of 1 anterior cerebral artery is expected to cause
bilateral CBF-increases in the ACA-supplied brain regions as well as partly in the MCA-territory via anastomoses.

In contrast to marked CBF elevations in the nonischemic
brain, we did not observe significant increases in the ACA
territory the ischemic brain. Further, only a marginal
improvement of ipsilesional CBF within the ischemic core and
the penumbra were noted. These results corroborate the
findings from a previous study showing greatly attenuated
CBF responses during SPG stimulation,\textsuperscript{9} likely a result of ischemia-induced (sub)maximal vasodilation in the ischemic
tissue with an attenuated capacity of the vessels to further
dilate in response to SPG stimulation. Abrupt vascular
occlusion such as in the model used can exhaust the capacity
of the collaterals to sustain CBF to the hypoperfused brain
areas,\textsuperscript{19} and hence these vessels may also “lose” their ability
to respond to vasodilating stimuli. Because stimulation-
induced CBF elevations were predominantly a result of ACA-vasodilation, it is likely that exhaustion of collaterals
(azygos anterior cerebral artery) prevented bilateral CBF-
increases in the ischemic brain.

Despite the fact that SPG stimulation induced some
improvement of core as well as penumbral CBF, this was
insufficient to significantly improve the threshold-defined
CBF lesion volume und arguably may have contributed
to—but was not the sole cause of—the impressive preserva-
tion of the ADC/CBF-mismatch as well as the neuroprotec-
tion seen on 24-hour TTC staining. Several potentially
interacting phenomena may have contributed to those bene-
ficial effects. First, because regional CBF was measured
within an area comprising only a fraction of the threshold
defined CBF lesion volume, a local steal phenomenon may
have caused CBF declines in other, noninvestigated areas,
rendering the overall CBF lesion volume unchanged. How-
ever, in light of the observed nonsignificant (\textasciitilde 10\%) reduc-
tion in CBF lesion volume it seems more likely that the
regional improvement in CBF was insufficient to affect the
total lesion volume. Second, continued stimulation may

\textbf{Figure 4.} Region specific cerebral blood flow (CBF) and apparent diffusion coefficient (ADC) values within investigated ipsilesional ROIs of nonstimulated (A and B, respectively) and SPG-stimulated (C and D, respectively) pMCAO-groups. Dotted lines denote critical viability thresholds. Within each contrallesional ROI, CBF and ADC values did not differ significantly between time points and were averaged for visual clarity in the figure (Contra). *$P<0.05$ vs nonstimulated controls (Student t test). ‡$P<0.05$ vs 30 minutes (RM-ANOVA).
induce subtle alterations of the BBB with subsequent vaso-
genic edema, which may have masked cytotoxic ADC de-
clines (“pseudo-normalization” of ADC). However, this pos-
sibility is remote because we did not observe any significant
ADC changes within ipsilesional and contralesional ACA-
cortex over time or between hemispheres, and our prelimi-
inary results demonstrated preserved BBB integrity with the
same stimulation paradigm. Nevertheless, to ascertain very
subtle alterations of BBB-integrity inaccessible by the meth-
odology used, future studies should assess permeability or
water content to further address this issue. Third, though
unexpected, our results may not be implausible in light of
several previous reports indicating that electrical stimulation
of parts of the brain could provide central neurogenic neuro-
protection independent of CBF-improvement.20,21 Proposed
mechanisms include decreased inflammation,22 spreading
depression,23 and inhibition of apoptosis.24 For example, it
was hypothesized that fastigial nucleus stimulation could
render cerebral vessels less responsive to the proinflamma-
tory action of interleukin-1β by overproduction of inhibitor
of nuclear factor κB-α (IkB-α), thus reducing the expression
of inducible nitric oxide synthase (NOS-2) and intracellular
adhesion molecule 1 (ICAM-1), and hence, attenuate infiltr-
ation of immune cells.22 Additionally, penumbral apoptosis
may be attenuated by modification of potassium channels and
increased tolerance to depolarizing stimuli23 as well as reduc-
tion of caspase-3 activity by reduced mitochondrial
function.24 However, it remains uncertain as to whether SPG stimulation provides stimulation through simi-
lar pathways. Nevertheless, given that SPG stimulation in the
nonischemic brain causes vasodilation through release of
NO,6,7 it is tempting to speculate that this may play a crucial
role in the observed neuroprotection effect. NO can freely
diffuse to adjacent cells and is a multimodal endogenous
mediator that can prevent inflammation, oxidative damage,
thrombosis, apoptosis, and platelet aggregation as well as
improvement of neovascularization and mobilization of stem
and progenitor cells.8 Importantly, administration of NO in
models of permanent cerebral ischemia demonstrated neuro-
protective properties leading to a reduction in final infarct
size.9 However, future studies should elucidate the precise
neuroprotective mechanisms underlying SPG stimulation and
examine its effects on complex functional outcome measures.
For example, involvement of NO could be confirmed or ruled
out by using a nitric oxide synthase inhibitor. Lastly, our
findings warrant a lengthier observation period in a less
severe ischemia model that may enhance the ability to show
whether the tissue protection observed by MRI and brain
pathology persists chronically. The importance of better
understanding the ameliorative effects associated with SPG
stimulation in ischemic stroke is highlighted by the recent
development of an orally, minimally invasive implantable
device (NeuroPath, BrainsGate LTD) that is currently under
investigation for ischemic stroke treatment within an interna-
tional multicenter pilot study.

Summary
In conclusion our results demonstrate that SPG-stimulation
(1) bilaterally elevates CBF within the normal brain, (2)
acutely preserves the CBF/ADC mismatch largely indepen-
dent of altering CBF, and (3) reduces infarct size in the rat
permanent suture model. These observations may be clini-
cally relevant as only a small minority of clinical stroke
patients are currently eligible for reperfusion therapy and
thrombolysis is often delayed or incomplete.

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Disclosures
M.F. is a member of the advisory board and N.H. a consultant for
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