Effects of Noninvasive Facial Nerve Stimulation in the Dog
Middle Cerebral Artery Occlusion Model of Ischemic Stroke

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Background and Purpose—Facial nerve stimulation has been proposed as a new treatment of ischemic stroke because autonomic components of the nerve dilate cerebral arteries and increase cerebral blood flow when activated. A noninvasive facial nerve stimulator device based on pulsed magnetic stimulation was tested in a dog middle cerebral artery occlusion model.

Methods—We used an ischemic stroke dog model involving injection of autologous blood clot into the internal carotid artery that reliably embolizes to the middle cerebral artery. Thirty minutes after middle cerebral artery occlusion, the geniculate ganglion region of the facial nerve was stimulated for 5 minutes. Brain perfusion was measured using gadolinium-enhanced contrast MRI, and ATP and total phosphate levels were measured using 31P spectroscopy. Separately, a dog model of brain hemorrhage involving puncture of the intracranial internal carotid artery served as an initial examination of facial nerve stimulation safety.

Results—Facial nerve stimulation caused a significant improvement in perfusion in the hemisphere affected by ischemic stroke and a reduction in ischemic core volume in comparison to sham stimulation control. The ATP/total phosphate ratio showed a large decrease poststroke in the control group versus a normal level in the stimulation group. The same stimulation administered to dogs with brain hemorrhage did not cause hematoma enlargement.

Conclusions—These results support the development and evaluation of a noninvasive facial nerve stimulator device as a potential treatment of ischemic stroke. (Stroke. 2014;45:1102-1107.)

Key Words: autonomic nervous system ■ facial nerve ■ perfusion ■ vasodilation

The currently available emergency treatments for ischemic stroke focus on removing the occlusive blood clot. However, another means for improving cerebral blood flow (CBF) in ischemic stroke is available, namely, dilating the cerebral arteries. Numerous animal studies have demonstrated that facial nerve stimulation with electric current dilates cerebral arteries and increases CBF (reviewed in Borsody et al1). Additional studies have shown that electric stimulation of autonomic branches of the facial nerve improves ischemic stroke measures2,3 and that lesions of those branches worsen ischemic stroke severity.4,5 However, accessing the facial nerve in the clinical setting so as to apply electric current to it would be difficult.

Previously, we demonstrated that noninvasive stimulation of the facial nerve trunk with magnetic energy in normal dogs and sheep increases CBF and causes vasodilation of cerebral arteries.1 Herein, we report on the effect of such stimulation in a clinically relevant dog ischemic stroke model. Additionally, we assess the effect of the same stimulation administered to dogs with brain hemorrhage caused by puncture of the intracranial internal carotid artery (ICA) as a test of safety of this potential treatment.

Methods

Ethical Approval and Facial Nerve Stimulation

All experimental procedures were approved by the Ethics Committee of the Universidad Autónoma Metropolitana of Mexico City. Facial nerve stimulation was performed with a modified transcranial magnetic stimulator (MagPro R30; MagVenture, Atlanta, GA) equipped with a fluid-cooled 6.5 cm figure-8 stimulation coil. Stimulation power was set at 1.8 T at the coil surface. Stimulation was administered with 280 μs biphasic pulses delivered at 10 Hz for a 5-minute period in all experiments. Placement of the stimulation coil was performed as described in Figure I in the online-only Data Supplement.

Ischemic Stroke Experiments

A total of 12 adult mongrel dogs weighing 15 to 37 kg were kept with ad libitum access to food and water until 8 hours before the
experimental procedure, at which time they were food-restricted. Dogs were randomly assigned to either stimulation (n=6) or control (n=6) groups. Anesthesia was induced with intramuscular injection of Zoletil (tiletamine/zolazepam, 1:1; 7 mg/kg), propofol (2.5 mg/kg), and fentanyl (2 μg/kg), and anesthesia was maintained with propofol (10 mg/kg per hour). Dogs were intubated after induction and mechanically ventilated throughout the experiment. Vital sign monitoring included heart rate, blood pressure, and arterial blood gas measurement. Ventilation was titrated according to oxygen saturation and end-tidal CO₂ levels.

Middle cerebral artery (MCA) occlusion was induced in all 12 animals by the injection of an autologous blood clot into an ICA through an endovascular catheter under digital subtraction angiography. After clot injection, MCA occlusion was confirmed by digital subtraction angiography, MR angiography, and loss of CBF in the MCA distribution on baseline imaging. In 1 dog allocated to the stimulation group, no loss of CBF was identified despite MCA occlusion on digital subtraction angiography and MR angiography; this dog was excluded from further analysis. At the end of each experiment, dogs were euthanized by intravenous injection of potassium chloride under general anesthesia.

Detailed descriptions of MRI protocols are available in the online-only Data Supplement. Tissue perfusion was assessed using standard blood flow thresholds for large animal stroke models. CBF and tissue perfusion were calculated by computer algorithm; blinding of images was not possible because of pronounced flush of blood to the extracranial tissues that identified stimulated animals (Figure 1). Average normalized perfusion index, ischemic core volumes, and ATP/total phosphate ratio of the stimulation group were compared with those of the control group with repeated-measures ANOVA (NCSS software package), using data sets beginning at the poststroke time point. Based on the expectation of increasing CBF with facial nerve stimulation, 1-sided tests were used.

Brain Hemorrhage Experiments

Three dogs as described above were used in these experiments. Intracranial hemorrhage was induced by puncture of the ICA with a Touhy needle advanced through a frontal craniotomy under neuronavigation guidance. On successful rupture of the ICA, intracranial hematoma was monitored with T1 and T2 MRI every 15 minutes until it was judged stable by the study staff on 3 consecutive imaging studies, after which facial nerve stimulation was delivered as described above. Follow-up T1 and T2 MRI and perfusion imaging were then performed immediately (t=0), 30 minutes, and 60 minutes poststimulation. Hematoma size as demonstrated on T1 and T2 imaging was judged qualitatively by the study staff that included a neurologist (M.K.B.) and a neurosurgeon (F.C.P.).

Results

Effect of Facial Nerve Stimulation in Dogs With Ischemic Stroke

Five minutes of facial nerve stimulation was administered beginning 30 minutes after confirmation of MCA occlusion by angiography and poststroke MRI evaluation. Facial nerve stimulation with pulsed magnetic energy did not cause nystagmus in any animal during or after stimulation, nor was salivation or lacrimation manifestly increased in stimulated animals. Facial nerve stimulation did not affect vital signs (Table I in the online-only Data Supplement).

Figure 1. A, Effect of facial nerve stimulation on cerebral blood flow (CBF; perfusion index) in a dog with right middle cerebral artery (MCA) occlusion caused by an autologous blood clot. Stimulation (1.8 T biphasic pulses administered at 10 Hz for 5 min) was administered 30 min after confirmation of MCA occlusion. B, CBF after sham stimulation in a sham stimulation dog subject to left MCA occlusion. Coronal section at the level of the ear. Yellow outline encloses brain.
Figure 1 shows an example of perfusion images in representative dogs from both stimulation and control groups. CBF as measured by perfusion index was increased in the region of ischemia when stimulation was administered 30 minutes after MCA occlusion. The effect of facial nerve stimulation was durable, lasting ≥90 minutes poststimulation. No such increase in CBF in ischemic brain region was observed in control animals. In all dogs receiving facial nerve stimulation, blood flow to extracranial tissues was also increased, although this effect was only observed immediately poststimulation (t=0). No movement of mastication muscles was observed during stimulation, indicating that the activation of trigeminal motor system did not account for increased blood flow to or through extracranial tissues.

Figure 2 shows the group average effect of facial nerve stimulation on CBF (perfusion index), comparing stimulation and control groups. Average CBF was decreased to ≤70% of baseline levels in the ischemic hemisphere region of interest, and perfusion stayed at those depressed levels in the control group, whereas it was returned to normal by facial nerve stimulation (P<0.01). Improvement in CBF after facial nerve stimulation was found to be durable for ≥90 minutes poststimulation. CBF was not reduced in surrounding brain regions after stimulation, including nonischemic frontal, parietal, and occipital regions both in ipsilateral and contralateral hemispheres (Figure II in the online-only Data Supplement).

Facial nerve stimulation also reduced ischemic core volume (Figure 3). The reduction in ischemic core volume after facial nerve stimulation did not come at the expense of an enlargement of penumbral volume, and the size of ischemic core was statistically smaller in the stimulation group in comparison to the control group (P<0.01) that showed an enlargement of ischemic core volume over time. By the 90-minute time point, however, it appeared that ischemic core volume began to increase in the stimulation group.

Effect of Facial Nerve Stimulation in Dogs With Brain Hemorrhage

Puncture of intracranial ICA with a Touhy needle did not kill any of the 3 dogs subjected to the procedure, and it produced a mixture of subarachnoid hemorrhage with intraventricular and intraparenchymal extension. Using the same parameters administered in ischemic stroke experiments, facial nerve stimulation ipsilateral to arterial puncture in dogs with stable intracranial hematomas did not kill any of the dogs, nor did it cause gross enlargement of the hematoma (Figure 5). CBF did not seem to change from baseline in this small group of dogs, and in fact, a large decrease in blood flow to extracranial tissues was observed (compare bottom panels of Figures 5 with immediately post-stimulation panel in Figure 1A).

Discussion

We used the dog model of embolic MCA occlusion with autologous blood clots in our experiments because it resembles the clinical condition of human embolic stroke. Autologous blood clot was injected into distal ICA from where it was carried by the blood flow into cerebral arteries; typically, the clot lodges in the MCA, but the precise site of occlusion cannot be controlled.8,9 As a result, the degree of loss of CBF, involvement of anterior communicating and cerebral arteries with MCA occlusion, and size and location of the infarct can exhibit considerable variability among the dogs, which is similar to the clinical reality of ischemic stroke. However, we did not experience much of this variability. Instead, we noted the failure of complete MCA occlusion to result in any region of reduced CBF on contrast-enhanced perfusion MRI in 1 dog—the likely effect of robust collateralization around the proximal MCA in this species.10

Despite the variability of the model, the effect of noninvasive magnetic stimulation of facial nerve was evident and statistically significant with only 11 experimental subjects. We demonstrate that facial nerve stimulation increased CBF when administered 30 minutes after MCA occlusion, and the CBF response outlasted the 5-minute stimulation period by ≥90 minutes after cessation of stimulation. The improvement in CBF in the region of interest in ischemic hemisphere was not attributable to a steal of blood from surrounding brain regions, which, to the contrary, exhibited a small degree of increased CBF themselves, consistent with a diffuse vasodilation of arteries of anterior circulation in response to facial nerve stimulation.11
The improvement in CBF after facial nerve stimulation translated into a reduction in ischemic core volume. Over a period of several hours, the volume of ischemic core is expected to increase as tissue in the penumbral zone succumbs to prolonged ischemia, but the acute time frame of our experiments may not have allowed the full area of hypoperfusion to manifest because both ischemic core and penumbral volumes increased in the control group. Indeed, within the 90-minute post–sham stimulation time frame in the control group, the total area of hypoperfusion seems to be reaching a plateau. In comparison, the group subjected to facial nerve stimulation demonstrated a significant reduction in ischemic core volume. This change may reflect the shifting of tissue satisfying an ischemic core definition to slightly improved perfusion, leading it to be classified as ischemic penumbra, and similarly the shifting of ischemic penumbra to normal perfusion; such a change would be consistent with a global improvement in perfusion or with a robust network of collateralization that can feed an ischemic brain region from multiple sides. The latter, in fact, seems to be available to the MCA in dogs.

Tissue perfusion measures further suggest that the effect of facial nerve stimulation may be temporary, because the volume of ischemic core began to increase in the stimulation group 90 minutes after stimulation. This may reflect reocclusion of the MCA after gradual loss of a vasodilatory drive from the facial nerve, although the CBF response did not seem to weaken at that time point. However, tissue perfusion in ischemic core may not have been fully captured by the hemispheric region of interest used to measure CBF, allowing the 2 measures to be in discordance. If vasodilation in affected portions of the MCA were to weaken faster than in unaffected arteries of the brain, reinstitution of ischemic core could occur while CBF outside of ischemic core region remains elevated.

But we do not view this data and question why the effect of facial nerve stimulation only lasts between 60 and 90...
minutes; rather, we are curious about how the effect of 5 minutes of facial nerve stimulation persists for as long as it did in our experiments. In normal (nonstroke) animals, Goadsby applied direct electric stimulation to the facial nerve in posterior fossa, and by applying 1 minute of continuous stimulation at 10 Hz, the increase in CBF began to return to baseline immediately after cessation of stimulation. Inconsistent evidence exists that facial nerve stimulation may have longer effects in animals with ischemic stroke than in normal animals. In 1 study of rats with permanent MCA occlusion published only in abstract form, direct electric stimulation of the sphenopalatine ganglion (which receives some of the vasoactive petrosal branches from the facial nerve) for a total of 10 minutes was reported to increase CBF by >40% for ≥24 hours. However, in another study in the same rat model, a total of 8 minutes of otherwise comparable sphenopalatine ganglion stimulation produced a notably smaller increase in CBF that returned to baseline within a matter of few minutes. In our experiments with noninvasive magnetic facial nerve stimulation, we observed increases in CBF lasting 20 to 30 minutes after a 5-minute stimulation period in normal sheep and dogs that were dependent on targeting the geniculate ganglion region of the facial nerve. We think that the longer effect of stimulation seen in our ischemic stroke experiments may reflect either persistent vasodilation or dissolution of the blood clot, and indeed, vasodilation may facilitate breakdown of blood clot by the physical force of partially restored blood flow. Spontaneous lysis of occluding clot in some dogs, but not all, might explain the suddenly increased variability observed in ischemic core volumes in dogs 90 minutes after stimulation. These questions will be addressed by upcoming experiments.

The beneficial effects of facial nerve stimulation on CBF and tissue perfusion are further corroborated by the ATP/total phosphate ratio, a commonly used measure of energy state of brain tissue. In contrast to the decrease in ATP/total phosphate ratio in the control group, which implies loss of critical energy reserves, no such decrease was observed after stimulation of the facial nerve. In fact, a small increase in the ratio was observed. Because we see no parallels for this overshoot in the scientific literature, we think it represents nothing more than the variability in the measure. However, we must recognize that a significant volume of brain was still ischemic core at the time of the ATP/total phosphate ratio overshoot, and the only way to explain the coincidental observations would be to presume the overshoot to be real if not underestimating the energy state of noncore brain. The preservation of ATP stores is consistent with improvement of CBF and tissue perfusion that reduced the volume of ischemic core.

We suggest that a facial nerve stimulator using pulsed magnetic energy directed at the facial nerve in the temporal bone would be safe and tolerable when used in humans. Allowing for its application in anesthetized animals in this report, facial nerve stimulation did not seem to have many of the potential adverse effects that would be expected based on the site of stimulation. No nystagmus nor excessive salivation or lacrimation were noted during or after stimulation, an observation confirmed in our normal animal experiments. We consider the absence of nystagmus an important finding, because the placement of

Figure 5. Effect of facial nerve stimulation on stable hematoma volume after internal carotid artery rupture. Top: T1 MRI demonstrating subarachnoid and intraventricular hemorrhage immediately before stimulation (left) and 60 min after stimulation with parameters described in Figure 1. Bottom: Perfusion image of blood flow to the brain (yellow outline) and extracranial tissues immediately poststimulation.
stimulation coil over the ear interposes the vestibular apparatus of the inner ear in the pathway of magnetic field. Other aspects of neurological examination were not available in anesthetized, nonsurvival dog subjects, notably hearing, because the auditory structures of inner ear are similarly in the path of magnetic field. However, previous experiments did recover several dogs from the experimental procedure that had involved facial nerve stimulation with comparable parameters, and in those cases, no evidence of neurological impairment was observed by the study veterinarian. Furthermore, we have conducted first-in-man experiments in an unsedated healthy volunteer and found no vertigo, nystagmus, tinnitus, or pain during or after stimulation. Additional testing in normal subjects is warranted and planned for 2014. In these normal subject studies, stimulation coil designs that more specifically stimulate the facial nerve (versus surrounding structures) will be used so as to reduce the likelihood of side effects and magnetic field exposure to the brain.

Remarkably, pulsed magnetic stimulation of the facial nerve also appeared safe in dogs with brain hemorrhage, causing no fatalities in a small group of dogs and no enlargement of hematoma size after stimulation. Interestingly, perfusion imaging qualitatively demonstrated a pronounced decrease in extracranial blood flow in such animals and no difference in CBF versus baseline. These surprising findings may reflect an inherent property of facial nerve function because the stimulation parameters used were identical to those in ischemic stroke experiments. We currently have no mechanistic explanation for this observation, but we hypothesize that the autonomic components of the facial nerve are subject to inhibitory regulation triggered by increased intracranial pressure or blood products. Such regulation may be provided by the trigeminal nerve, which has sensory innervation of the meninges that is known to be responsive to various irritants and distortion of the dura and which connects to the brain stem nuclei from which vasoactive components of the facial nerve derive. Further studies into this curious finding are necessary to define its mechanism.

Conclusions

Based on the results of experiments in dogs, we think that a medical device based on pulsed magnetic stimulation of the facial nerve could be beneficial in the emergency treatment of patients with ischemic stroke based on the noninvasive nature of stimulation, general safety of stimulation, and prolonged and sizable effect on CBF and tissue perfusion seen after a relatively short stimulation period. In conjunction with further animal testing, we are now proceeding with the development of a clinical prototype for assessing safety and efficacy in humans.

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Disclosures

Drs Borsody and Sacristan are employed by, and shareholders in, Nervive Inc, which owns patents on the facial nerve stimulator. The other authors have no conflicts to report.

References

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SUPPLEMENTAL MATERIAL

Manuscript Title: Effects of non-invasive facial nerve stimulation in the dog middle cerebral artery occlusion model of ischemic stroke.
Supplemental Methods

Animals and Baseline Imaging

Dogs specially bred and maintained for research purposes were obtained from the animal facility center of the Benemerita Universidad Autónoma de Puebla and transported to the laboratory on the experiment day. All dogs were subject to veterinarian prescreening to ensure full health prior to experimental use.

Mongrel dogs were used instead of beagles because of their larger head sizes, which as a proportion of the stimulation coil size is closer to a human facial nerve stimulator currently in development. Additionally, it is the experience of some laboratories that the embolism procedure appears to be more reliable in larger dogs [1].

All dogs were strapped in a lateral recumbent position to a specially-designed board so that they could be transferred in and out of the MRI scanner and always be placed in the same position within the scanner. Baseline MRI scans were taken including reference T1-weighted and T2-weighted images, MR angiography, contrast-enhanced perfusion, and 31P spectroscopy of the brain (ischemic stroke experiments only).

Neuronavigation Procedure

Prior to baseline imaging, a series of 6 fiducial markers (vitamin E capsules) were glued on to different locations on the head. The fiducial markers were readily identified on T1 MRI. A 3-D reconstruction of the dog’s head was created for matching the spatial coordinates defined by the fiducials to the coordinates for the stimulation target identified on T2 MRI; a registration procedure using a pointer equipped with a position transducer (Brain Science Tools, Utrecht) created the match. A second position transducer on the stimulation coil guided coil positioning relative to the head so that the coil could be placed in a manner directing its center point (i.e., the point of maximum magnetic field strength) at the desired target, namely the geniculate ganglion region of the facial nerve. The stimulation coil was held in position against the dog’s head by a locking mechanical arm.

The geniculate ganglion region of the facial nerve was identified on MRI based on its position anterior and superior to the semicircular canals, lateral and inferior to the cochlea, and medial to the ear canal. The ear canal was filled with water-soaked cotton to assist with imaging (Figure I).

Autologous Blood Clot Preparation and Injection, and Post-Stroke Imaging

Two days prior to experimental use, arterial blood was drawn by venipuncture and used to form blood clot. Clots were prepared by taking 20 mL of blood in 1 mL syringes and, after 2 hours at room temperature, storing them at 4°C for 48 hours. This procedure produced the most compact clots [2]. Before use, the clots were removed and repeatedly washed in a Petri dish containing
phosphate-buffered saline (PBS) and transferred into a second Petri dish containing PBS until use. Clots of firm consistency were drawn into a syringe attached directly to the catheter placed in the ICA and the clots were gently injected. Typically 2-3 clots totalling about 0.3 -0.5 mL volume were injected to obtain complete occlusion of the MCA.

Blood clots were injected using an intravascular catheter (0.035") introduced via the femoral artery under X-ray angiography by an experienced clinical neurointerventionalist (FCP). The catheter was advanced into the distal internal carotid artery so as to bring the tip as close as possible to the origin of the MCA. For this reason, the side of blood clot injection was left to the discretion of the neurointerventionalist and the vascular anatomy of the individual dog, and the majority of blood clots were injected on the right side.

Following injection of blood clot into the distal ICA and occlusion of the MCA, the dogs were transferred back to the MRI scanner to repeat the reference, perfusion, angiography, and \(^{31}\)P spectroscopy scans. Then, the dogs were again removed from the scanner and subject to facial nerve stimulation (stimulation group) or else handled similarly without the delivery of stimulation to the facial nerve (sham stimulation control group), which was initiated 30 minutes after confirmation of the arterial occlusion and loss of CBF. Immediately following stimulation or sham stimulation the animals were returned to the MRI scanner. Less than 30 seconds was required to transfer the animal from the stimulation table into position in the MRI scanner. After stimulation, imaging studies were obtained immediately after stimulation (t=0), 30 minutes after stimulation, 60 minutes after stimulation, and 90 minutes after stimulation.

**Brain Hemorrhage Surgical Procedure**

A 1 cm\(^2\) craniotomy over the frontal cortex was created with a surgical drill and a 20-gauge Touhy needle was advanced through the forebrain toward the ICA under neuronavigation guidance. Puncture of the ICA was confirmed by pulsatile backflow of bright red blood through the Touhy needle, at which time the needle was removed and the craniotomy was closed with a block of excised temporalis muscle. The scalp incision was sutured and the animal was then evaluated with T1 and T2 MRI every 15 minutes until the intracranial hemorrhage was judged to be of stable size by the study team on three consecutive images. At that point, the animal was removed from the MRI and subject to facial nerve stimulation using neuronavigation positioning of the stimulation coils. Following stimulation, the animal was returned to the MRI and evaluated immediately, 30 minutes, and 60 minutes afterward with T1 and T2 imaging.

**MRI Protocols**

The primary objective of this study was to demonstrate that magnetic stimulation of the facial nerve improves perfusion of ischemic brain. For this purpose we used contrast-enhanced perfusion MRI, a well-established technique for detecting and characterizing ischemic brain tissue [3,4].

MRI scans were obtained using a Philips Achieva 3T MRI scanner and an 8 channel SENSE® head coil. MR angiography used a 3D Phase Contrast Angiography sequence with a FOV of 150
mm centered so that the bifurcation of the carotid artery could be seen as well as the cerebral arteries and the circle of Willis. For the perfusion and the T2 reference scans, the same geometry was used in order to facilitate registration, with 25 coronal slices spanning the entire brain with a 230 mm FOV. T2 imaging used a multi-slice Fast Spin Echo sequence. Perfusion imaging used the Philips PRESTO® sequence consisting of 40 dynamic phase contrast images per slice repeated every 1.6 seconds, initiated after an 8 cc bolus of contrast agent (gadopentate dimeglumine 0.5 mmol/ml) and a 15 cc saline flush.

For CBF measurement, perfusion index maps were calculated using the PRESTO Philips software. Twenty five maps were calculated for each slice during each perfusion scan. For analysis we focused on only slices number 10 to 14, corresponding to the center of the brain where the ischemia was reliably located. T2 images were used to create a mask and identify and segment brain tissue. We defined two symmetrical square 20 x 20 mm regions of interest (ROIs), anchored to the same anatomical feature in the center of the brain, for both the stroke side and the non-stroke / contralateral side for each of these five slices. Then, we calculated the average perfusion index at each time point for the tissue within these slices. To eliminate variations from scan to scan and from subject to subject, we normalized the perfusion index on the stroke side to that of the non-stroke / contralateral side.

In the short time frame we chose to study, we found that DWI signal strength was insufficient for defining the ischemic region; a minimal DWI signal in this timeframe has been confirmed in other studies of the dog ischemic stroke model [4]. In the autologous blood clot embolism dog model, growth of DWI lesion volume (which some believe is a reliable measure of the ischemic core [5]) has been demonstrated to occur within a 2 hour timeframe [3], but other embolic dog models suggest that the appearance of significant DWI signal within 1 hour would be uncommon [6].

Tissue perfusion was assessed using standard blood flow thresholds for large animal stroke models [7,8]. We segmented the brain into three volumes: normally perfused tissue (> 24 mL / 100 g tissue / min), penumbra (8-24 mL / 100 g tissue / min), and ischemic core (< 8 mL / 100 g tissue / min). The mask created from T2 images was again employed to isolate the brain, which was analyzed in its entirety (no ROIs were used for this analysis). Slices 10-14 were analyzed in this manner and the volume corresponding to ventricles and the subarachnoid space was subtracted from these measures.

$^{31}$P MR spectroscopy were obtained using ISIS single voxel localization (voxel size of 5 cm (AP) x 2.5 cm (RL) x 5 cm (FH)) located in the ischemia-affected hemisphere and with four echo trains with an effective echo time of 95.6 msec and a repetition time of 4.5 sec, repeated 128 times for a total acquisition time of 9.6 minutes. Spectral processing was performed using Philips spectroscopy software to curve fit and estimate the area under the ATP ($\alpha, \beta, \gamma$), phosphocreatinine, and inorganic phosphate peaks. The $^{31}$P spectroscopy scans required a change of radiofrequency coil and were performed after the baseline acquisition, the post-stroke acquisition, and the 60 and 90 minute post-stimulation acquisitions.
Table I: Vital Sign Measurements Before and After Facial Nerve Stimulation. Mean ± SEM. Insufficient data was available at the 90 minute post-stimulation time point. Group comparisons with t-tests corrected for multiple comparisons failed to show any statistically-significant differences.
Supplemental Figures

Figure I: Neuronavigation procedure.
Figure II: Perfusion index from regions surrounding the ischemic area, comparing post-stroke / pre-stimulation versus 30 min post-stimulation time points. Mean ± SEM.
**Figure III:** Effect on CBF as measured by average perfusion index in the ischemic hemisphere relative to the contralateral hemisphere, with the baseline measure set as 100%. Blue line = stimulated group (n=11 dogs); red line = control group (n=6 dogs). Stimulation for 5 minutes was administered 30 minutes (n=10 dogs) or 90 minutes (n=1 dog) after confirmation of MCA occlusion. P < 0.05 between groups, repeated measures ANOVA. Mean ± SEM.
Figure IV: Effect of facial nerve stimulation on ischemic penumbra and core volumes in stimulated dogs (n=11) and control dogs (n=6). Standard perfusion thresholds were used to categorize brain tissue [7,8]: ischemic core (red) < 8 mL / 100g / min; penumbra (yellow) = 8-24 mL / 100g / min; normal tissue (green) > 24 mL / 100g / min. Stimulation for 5 minutes was administered 30 minutes (n=10 dogs) or 90 minutes (n=1 dog) after confirmation of MCA occlusion. P < 0.05 between groups, repeated measures ANOVA. Mean ± SEM.
Supplemental References


