Magnetic Facial Nerve Stimulation in Animal Models of Active Seizure

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Abstract

Purpose: As part of our efforts to develop a non-invasive facial nerve stimulator as an emergency treatment for ischemic stroke, we considered possible safety consequences if the technology was misapplied to stroke mimics, e.g., seizure. We hypothesized that magnetic facial nerve stimulation would worsen epileptiform activity in two animal models of active seizures. The rat intraperitoneal kainate model and pig intracortical penicillin model were employed. Magnetic facial nerve stimulation was delivered unilaterally at a variety of stimulation parameters, and the effect on ictal epileptiform activity measured by electroencephalography was determined according to an established categorical scale.

Principal Results: In 6 rats and 3 pigs evaluated with 83 stimulation trials, only a single stimulation trial was associated with worsening epileptiform activity according to a standard categorization scheme. Surprisingly, a reduction in the severity of the epileptiform activity was observed in 20 of 50 stimulation trials using a theta burst pattern (3 pulses at 30 Hz repeated at 0.5 - 10 Hz) versus 2 of 33 stimulation trials using simple monotonic patterns (P < 0.005, chi-squared test). The reduction of epileptiform activity after stimulation lasted a few minutes and was reproducible.

Major Conclusions: Epileptiform activity measured by electroencephalography was not reliably worsened by repetitive facial nerve stimulation with pulsed magnetic energy, even when
significant brain exposure to the magnetic field occurred as in the rat model. To the contrary, a temporary reduction in epileptiform activity was often, but not invariably, observed with certain stimulation parameters.
1. Introduction

We are developing a non-invasive facial nerve stimulator device as an emergency treatment for ischemic stroke. It is well-known that electric stimulation of the parasympathetic petrosal components of the facial nerve causes dilation of the cranial arteries and an increase in cerebral blood flow in normal animals [Forbes et al., 1937, 1939; Meyer et al., 1967; Adams et al., 1989; Goadsby, 1989, 1990a, 1990b, 1991, 1994; Sato et al., 1997; Toda et al., 2000a, 2000b] and in animals with brain ischemia [Yarnitsky et al., 2005, 2006; Henninger and Fisher, 2007; Takahashi et al., 2011]. Along those lines, we have shown that pulsed magnetic energy can activate this parasympathetic function of the facial nerve and cause a significant increase in cerebral blood flow both in normal animals [Borsody et al., 2013] and in the dog [Garcia et al., 2013; Borsody et al., 2014] and rabbit (data unpublished) ischemic stroke models. Indeed, a recently-completed normal subject safety and tolerability study also supports this observation.

However, the development of a magnetic facial nerve stimulator for the treatment of ischemic stroke creates potential safety concerns because of the non-specific stimulation of the nearby brain, particularly when that part of the brain is injured by stroke. Furthermore, increased blood flow appears to precede even the electrographic onset of some seizures [Jackson et al., 1994; Adelson et al., 1999], and so increasing cerebral blood flow by means of facial nerve stimulation could theoretically provoke seizure activity from injured brain tissue even when the tissue is not exposed to magnetic field. More generally speaking, transcranial magnetic stimulation of the brain, e.g., for the treatment of depression, has the long-standing safety concern of inducing
seizures [Wasserman, 1998] even though this concern does not appear to be clinically substantiated [Rossi et al., 2009]. Because of this potential safety concern, we undertook pilot preclinical experiments that applied magnetic facial nerve stimulation to animals with ongoing seizure activity.

2. Materials and Methods

Experiments in rat (n=6, 3 males and 3 females) and pig (n=3, all male) were conducted at the National Center for the Investigation of Imaging and Medical Instrumentation (CI3M) of the Universidad Autónoma Metropolitana of Mexico City with Institutional Animal Care and Use Committee (IACUC) approval. The ARRIVE guidelines were followed, where applicable.

In order to deliver facial nerve stimulation, it was necessary to anesthetize the animals so that they did not exhibit convulsive movements. This, in turn, necessitated the use of epileptiform activity on the electroencephalogram as a surrogate marker for motoric seizure activity. A scale for grading ictal epileptiform activity in sheep proposed by Opdam et al. [2002] was employed for assessing the severity of, and change in, epileptiform activity. Electroencephalogram tracings were evaluated in an unblinded fashion by one of the authors (MKB) who is a board-certified neurologist.

Facial nerve stimulation was achieved with 280 μsec biphasic pulses delivered at a stimulation power of 75% of the maximum output of the stimulus generator, which corresponds to a
magnetic field strength of 1.5 Tesla at the coil surface. This power was sufficient to activate the parasympathetic function of the facial nerve in our previous experiments with rabbits, dogs, and sheep, and the power is not scaled according to the body size of the experimental subject since nerve fiber activation thresholds are not dependent upon the size of the animal. Facial nerve stimulation was delivered with a commercially-available transcranial magnetic stimulator device (MagPro R30; MagVenture, Atlanta, Georgia) and a 6.5-cm mean diameter figure-8 stimulation coil (Cool B65). The stimulation coil was kept at surface temperatures < 40°C using a circulating fluid cooler equipped with a radiator.

2.1. Rat Experiment Protocol

The specific aim of the rat experiments was to determine if facial nerve stimulation with pulsed magnetic energy worsens the severity of epileptiform activity in a model of continuous and progressive seizure activity. The rat intraperitoneal kainate injection model [Friedman et al., 1994] was chosen because of the severe and irreversible nature of the seizure activity and because the magnetic field generated by the stimulation coil certainly exposes the animal’s brain to strong magnetic field, creating a very permissive evaluation of the safety of magnetic stimulation on seizure activity.

Sprague-Dawley rats (200-250 g bodyweight) were treated with intraperitoneal injection of kainic acid (15 mg/kg) and immediately subject to anesthesia with inhaled isoflurane (1.5 – 2.5% in 2 L/min compressed room air) titrated to the absence of withdrawal to hindpaw pinch. The animals were then placed in a lateral recumbent position on a foam frame. Electrodes fashioned
from stainless steel screws were secured into holes drilled in the skull; two active electrodes were placed 3-4 mm on either side of the coronal suture and 2 mm lateral to midline ipsilateral to the side of stimulation, and a reference electrode was secured immediately posterior to the lambda suture.

Electroencephalography in rats was performed with a Grass model 7D polygraph with wide band AC pre-amplifier model 7P5B (Grass Medical, Quincy MA). Filters were set a 1 Hz for the lower filter and 35 Hz for the high filter. When epileptiform activity became equivalent to Category 3 epileptiform activity according to the scheme of Opdam et al. [2002] (i.e., < 30 spikes per 30 seconds, no bursts), facial nerve stimulation trials were initiated. The stimulation coil was placed alongside the rat’s head after anesthetization so as to produce maximum movement of the facial musculature including platysma after single-pulse test stimulation; no neuronavigation was employed in the rat experiments given the animal’s small size.

A variety of stimulation parameters were employed in the experiments to determine how brief periods of stimulation affected epileptiform activity. Initially, stimulation parameters that were previously shown to be effective at increasing cerebral blood flow [Borsody et al., 2013, 2014; Garcia et al., 2013] were employed (10 Hz for 5 minutes). When those parameters were reliably found to not worsen the epileptiform activity, additional stimulation parameters were assessed including a theta burst pattern [Wischenewski et al., 2015] (Figure 1). Different stimulation parameters were administered only after an interval of at least 15 minutes in the rat experiments. However, in some experiments, the same stimulation parameters were repeatedly administered to determine if there was an additive effect of multiple stimulations.
Upon completion of the experiments, the rat was sacrificed by intraperitoneal pentobarbital injection.

2.2. Pig Experiment Protocol

The specific aims of the pig experiments was to confirm (1) that magnetic facial nerve stimulation does not worsen seizure activity in a second animal model and (2) that facial nerve stimulation can reduce epileptiform activity in a large animal model wherein the stimulation is more clinically-relevant in terms of anatomical specificity. Accordingly, we modified for use in pig a sheep model of penicillin-induced seizures [Opdam et al., 2002] that has good reproducibility, treatment effectiveness prediction, and clinical relevance.

Adult Yorkshire breed pigs were used; the accepted weight range was 15-35 kg. On the day of experimentation, pigs were induced with intramuscular azaperone (2 mg/kg) and ketamine (15 mg/kg). The pigs were intubated after induction and then isoflurane (1-2%) in 100% oxygen (3.2 L/min) was used for the maintenance of anesthesia under spontaneous respiration. A femoral artery catheter was placed to monitor blood pressure, heart rate, and arterial blood gases.

T1 images of the head and brain were obtained for use in neuronavigation and then a 2x2 cm square of skull was removed over the frontal cortex without penetrating the dura. Stainless steel wire electrodes were placed on the surface of the dura underneath the edge of the craniotomy, 2 cm apart, and a reference electrode was secured to the supraoccipital condyle with a stainless
steel screw. Then, between the electrodes, a 10 μL microsyringe was advanced through the dura and into the cortex to a depth of 1 mm. A dose of 16,000 IU of sodium penicillin (800 IU / μL in 0.9% normal saline) was infused over 2 minutes. Epileptiform potentials developed in all pigs within 30 seconds of penicillin injection and progressively worsened in severity as previously described [Opdam et al., 2002].

In order to place the stimulation coil under neuronavigation guidance, spatial positions were marked on 3-dimensional reconstructions of the T1 scan using readily-recognized landmarks of the pig’s head (i.e., outer edges of the orbits, teeth of the maxilla). Then, the positioning of the stimulation coil was determined relative to the 3-dimensional reconstruction of the T1 scan using a commercially-available neuronavigation system (NeNa; Brain Science Tools, the Netherlands) that recognizes the stimulation coil’s position in space relative to the pig’s head.

Electroencephalography was digitally recorded with a NeuroScan recorder (NeuroScan, El Paso, TX) at 1000 Hz sampling frequency and a bandwidth of 1-200 Hz. Once Category 3 epileptiform activity according to the scheme of Opdam et al. [2002] (i.e., ≥ 30 spikes per 30 seconds without bursts) was observed for a period of 2 minutes, facial nerve stimulation was administered. One stimulation trial was allocated to each set of parameters, and the stimulation trials were separated by 30 minutes.

Laser Doppler flowmetry was also performed in the pig experiments. A 1-cm burr hole was placed over the contralateral frontal cortex so as to avoid the craniotomy performed for electrode
placement. Superficial cerebral blood flow was measured using laser Doppler flowmetry probes (PF4001 main unit with PR407-1 probe), as previously described [Borsody et al., 2013]. Upon completion of the experiments, which lasted no more than 4 hours, the animal was sacrificed by intraarterial potassium chloride injection.

3. Results

No animal died during the course of these experiments, and epileptiform activity was reliably induced according to the established protocols we employed [Friedman et al., 1994; Opdam et al., 2002]. Prior to stimulation, we observed only a progressive worsening of epileptiform activity to a point comparable to Category 3 of Opdam et al.’s [2002] staging system; we did not observe any instance of spontaneous improvement in epileptiform activity.

The stimulation parameters employed in these experiments are shown in Table 1. In the rat experiments, 9 stimulation trials using parameters of 10 Hz for 5 minutes (which are those planned for use in the stroke treatment device) did not worsen ictal epileptiform activity. The range of parameters was then broadened. A total of 24 stimulation trials using monotonic rhythms for stimulation ranging from 0.5 – 20 Hz did not demonstrate any worsening in epileptiform activity. Similarly, patterned stimulation similar to theta burst patterns (3 pulses at 30 Hz repeated at 10 Hz) worsened epileptiform activity in only 1 of 47 stimulation trials (Figure 2), although the change persisted for at least 15 minutes and was not reproducible.
Interestingly, a reduction in epileptiform activity was often observed when stimulation was administered in theta burst patterns. Representative examples of this effect are provided in Figures 3-6 from both rat and pig experiments. The reduction in epileptiform activity was reliably transient, lasting a few minutes, and successive stimulations appeared to have a cumulative effect at reducing epileptiform activity (Figure 4 and 6). Comparing stimulation at 3 pulses at 30 Hz repeated at 10 Hz for any duration against all other stimulation parameters in the rat experiments showed a highly significant effect: 18 of 22 stimulation trials using theta burst patterns reduced epileptiform activity by at least one category level on the Opdam scale, versus only 2 of 31 trials with other parameters (P < 0.001, chi-squared test). The same comparison of the pig stimulation trials – numbering only 12 trials in total – demonstrated a trend toward improved epileptiform activity after stimulation with theta burst patterns (P = 0.07).

In the pig experiments, facial nerve stimulation did not affect blood pressure, heart rate, respiratory rate, or arterial blood gas measurements (data not shown). Furthermore, as measured by laser Doppler flowmetry, facial nerve stimulation at 10 Hz for 5 minutes increased cerebral blood flow (data not shown) whereas stimulation with the theta burst pattern did not increase cerebral blood flow (data not shown). Vital signs were not monitored in the rats.

4. Discussion
The facial nerve - which is best known for controlling the somatic musculature of facial expression – also has somatic sensory, special sensory, and autonomic functions. Parasympathetic fibers of the facial nerve ultimately conveyed to the cerebral arteries through the petrosal branches have the well-defined ability to increase salivation and lacrimation and also the little-known ability to dilate the arteries of the intracranial and extracranial head. Our aim is to use the ability of the facial nerve to dilate the cerebral arteries as the basis for a non-invasive emergency treatment for ischemic stroke based on pulsed magnetic stimulation of the nerve. The device we are developing focuses the magnetic field on the facial nerve trunk as it runs through the temporal bone, deep to the middle ear space, at a point where the parasympathetic fibers are still part of the nerve trunk and at a point where the trunk is closest to the surface of the brain. Nevertheless, a certain degree of non-specific exposure of the nearby brain (e.g., overlying lateral temporal lobe) is expected in man, and increased blood flow to the brain has the theoretic possibility of triggering seizure activity from damaged brain [Jackson et al., 1994], creating the safety concern that magnetic stimulation may cause or worsen seizure activity.

The specificity of the magnetic field is a limitation when assessing anatomical exposure to magnetic energy. It is, however, an inherent and even necessary feature of the technology we are developing as an emergency treatment of ischemic stroke, which must be rapidly placed on the head and quickly adjusted based only upon anatomical features (e.g., the ear canal). It is unclear what the activation potential is for magnetic energy in various neural tissues; accordingly, we cannot predict what structures are being activated by the magnetic stimulation in these experiments, although we can observe the physiological effects of those structures. In the rat experiments, it is very plausible – if not likely - that the magnetic field non-specifically activated
structures with antiepileptic effects. One such structure is the vagus nerve, the stimulation of which is an FDA-approved means of reducing seizure frequency [Connor et al., 2012] that can also interrupt ongoing seizure activity [Zeiler et al., 2015]. Non-specific stimulation of antiepileptic neural structures is less likely in the pig experiments: the target facial nerve in pig is offset by about 3 cm from the vagus nerve root at the brainstem in the posterolateral direction. Considering that magnetic field strength decays as the third power of distance, the magnetic field experienced by the vagal nerve would be quite small in the pig. Furthermore, we did not observe bradycardia suggestive of vagal nerve stimulation in the pig experiments. Another non-specific effect of magnetic facial nerve stimulation might be the activation of the cutaneous sensory innervation provided by the trigeminal nerve in the region around the ear and lateral surface of the head. While the orbital branches of the trigeminal nerve are being investigated as possible targets for neuromodulation of seizure activity with mixed results [Pack, 2013; Soss et al., 2015], the generalizability of the effect to other cutaneous branches of the trigeminal nerve is unknown. What is more, the distance between the facial nerve stimulation target and the orbital branches of the trigeminal nerve in the pig is almost certainly prohibitive.

Quite frankly, the concern that magnetic stimulation of the facial nerve will worsen seizure activity is not well substantiated by the medical literature or by electromagnetic physics. Most telling in this matter are the empiric demonstrations that pulsed magnetic stimulation does not trigger seizures from known epileptogenic foci in patients with epilepsy and the vanishingly uncommon reports of seizures that are unequivocally provoked by the application of transcranial magnetic stimulation of the brain [Tassinari et al., 2003; Rossi et al., 2009]. Furthermore, magnetic fields decay very rapidly as the third power of distance. While the proprietary design
of the stimulation coil for our facial nerve stimulator device (which was not used in these experiments) should reduce non-specific brain stimulation by at least 70% in man, we nevertheless undertook these pilot experiments to assess the safety of facial nerve stimulation in animals with active seizures using an off-the-shelf transcranial magnetic stimulation device as a worst-case scenario. On only 1 occasion in 77 stimulation trials did we observe an apparent worsening in ictal epileptiform activity after facial nerve stimulation. That worsening was not reproducible and was long-lasting to the point that it may have represented the expected progression of epileptiform activity inherent to the animal model [Opdam et al., 2002]. Indeed, with certain stimulation parameters, we much more commonly observed a reduction in epileptiform activity after magnetic facial nerve stimulation.

We hypothesize that the facial nerve may influence seizure activity as does the vagus nerve, and indeed the neuroanatomy of the facial nerve has important parallels to the vagus nerve: afferents of both nerves synapse in the solitary tract nucleus, the spinal trigeminal nucleus, and the dorsal horn of the cervical spinal cord [Contreras et al., 1980], which may then influence other brainstem centers such as the locus coeruleus and raphe nuclei that are thought to underlie the antiepileptic effect of vagal nerve stimulation [Krahl and Clark, 2012]. Similarly, the locus coeruleus and raphe nuclei that are thought to underlie the antiepileptic effect of vagus nerve stimulation may be involved in the facial nerve-induced cerebral artery dilation [Goadsby et al., 1984, 1985]. While there are no other reports of the effect of facial nerve stimulation on seizures in animal or man to our knowledge, clinical case series have demonstrated changes in electroencephalographic patterns after chronic facial nerve injury that would promote seizure development [Marzuoli et al., 1966], whereas physical irritation of the facial nerve affects the
electroencephalogram in a manner that would reduce seizure frequency [Oussova and Lerner, 1961]. In total, this limited literature and the observations we report here suggest that stimulation of the facial nerve may interrupt ongoing seizure activity.

Alternatively, fluctuations in the ictal epileptiform activity in our experiments may have given the appearance of improvement that was false attributed to facial nerve stimulation. Indeed, fluctuations in epileptiform activity may have been so significant that they mask a harmful effect of facial nerve stimulation on the epileptiform activity. In reality, spontaneous improvement in the epileptiform activity is highly unlikely in the rat and pig models we employed, which are otherwise described as “persistent seizure activity” [Friedman et al., 1994] and progressive through “a relatively predictable sequence” of epileptiform activity [Opdam et al., 2002]. To that point, we did not observe any improvement in epileptiform activity in any experiment while the epileptiform activity worsened to where facial nerve stimulation was administered (i.e., Category 3 severity according to the scale proposed by Opdam et al. [2002]. Indeed, the likelihood that the epileptiform activity spontaneously improved so as to give the false impression that facial nerve stimulation was responsible for the improvement, or so as to mask a worsening of the epileptiform activity caused by facial nerve stimulation, seems so unlikely that it would not be possible to call such improvements ‘spontaneous’. Spontaneous improvements in epileptiform activity becomes even less credible if it must be assumed that spontaneous improvement occurred repeatably only at the precise times when stimulation with theta burst patterns were administered.
The finding that facial nerve stimulation may reduce ictal epileptiform activity is a serendipitous finding for our laboratory and we are not prepared or qualified to pursue the observation further. We report these pilot study results so as to encourage further investigation conducted by other laboratories specialized in epileptology. In that effort we are ready to provide our assistance and collaboration.

5. Conclusions

Non-invasive magnetic facial nerve stimulation does not worsen epileptiform activity at stimulation parameters that increase cerebral blood flow. Instead, magnetic facial nerve stimulation may reduce the severity of ongoing epileptiform activity at certain stimulation parameters.
Acknowledgements and Funding

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Disclosure of Conflicts of Interest

Drs. Borsody and Sacristan are shareholders in, and serve in senior management positions on, Nervive, Inc., a for-profit company that is developing the facial nerve stimulation technology. The remaining authors have no conflicts of interest.
References


Figure 1: Graphical representation of biphasic pulsed magnetic stimulation at (A) a monotonic rhythm of 10 Hz, and (B) a theta burst pattern of 3 pulses at 30 Hz repeated at 10 Hz.

Figure 2: Stimulation trial in rat associated with an increase in epileptiform activity. Stimulation with theta burst parameters (3 pulses at 30 Hz repeated at 10 Hz for 5 minutes). The first stimulation appears to be associated with a worsening of the epileptiform activity that is not reproduced by the second stimulation 15 minutes later.

Figure 3: Stimulation trial in rat associated with a decrease in epileptiform activity. Stimulation with theta burst parameters (3 pulses at 30 Hz repeated at 10 Hz for 60 seconds).

Figure 4: Stimulation trial in rat associated with a decrease in epileptiform activity. Stimulation with theta burst parameters (3 pulses at 30 Hz repeated at 10 Hz for 60 seconds) administered singly and in 3 successive administrations. Timeline includes a 1 hour interval rest period.

Figure 5: Stimulation trial in pig associated with a decrease in epileptiform activity. Stimulation with theta burst parameters (3 pulses at 30 Hz repeated at 10 Hz for 60 seconds).
Figure 6: Stimulation trial in pig associated with a decrease in epileptiform activity. Stimulation with theta burst parameters (3 pulses at 30 Hz repeated at 10 Hz for 60 seconds) delivered in 2 successive administrations.
Table 1: Stimulation parameters employed in the rat and pig experiments. ↑ = improved one or more categories, ↓ = worsened one category according to the system of Opdam el al. [2002]; ↔ = no change in epileptiform activity category.

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* excludes 2 trials in 1 rat and 1 trial in pig that did not provide interpretable post-stimulation tracings due to movement of the electroencephalogram electrodes

** see Figure 2
Figure 1

3 pulses at 30 Hz
Figure 2

Stimulation #1
100μV
1 sec

Stimulation #2
baseline
30 sec post-stimulation
15 min post-stimulation
30 sec post-stimulation
Figure 3

Stimulation #1

Baseline
30 sec post-stimulation
2 min post-stimulation
5 min post-stimulation
10 min post-stimulation
30 min post-stimulation

100μV

1s
Figure 5
Figure 6

Baseline

Stimulation #1

30 sec post-stimulation

2 min post-stimulation

Stimulation #2

30 sec post-stimulation

2 min post-stimulation