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Magnetic facial nerve stimulation in animal models of active seizure

Mark K. Borsody[a,b,*], Andrea Garcia[c], Dawn M. Bielawski[a], Chisa Yamada[a,*], Emilio Sacristan[b,c]

[a] NeuroSpring, 35756 Foothills, Sterling Heights, MI 48312, United States
[b] Nervive, Inc., 526 S. Main St., Suite 801-A, Akron, OH 44311, United States
[c] National Center for the Investigation of Imaging and Medical Instrumentation, Universidad Autónoma Metropolitana, Mexico City, Mexico

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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 patterned stimulation (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,b, 1991; Goadsby and Hoskin, 1994; Sato et al., 1997; Toda et al., 2000a,b) 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.

* Corresponding author.
E-mail address: mborsody@hotmail.com (M.K. Borsody).

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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 μs 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 T 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. Anesthetic levels were kept constant throughout the experiment. 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 s, 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.

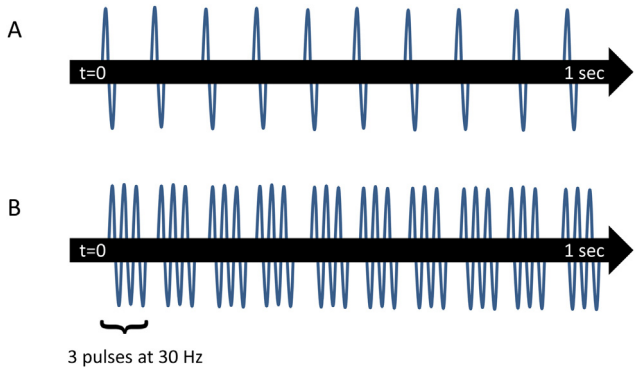

Fig. 1. Graphical representation of biphasic pulsed magnetic stimulation at (A) a monotonic rhythm of 10 Hz, and (B) patterned stimulation of 3 pulses at 30 Hz repeated at 10 Hz.

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 min). When those parameters were reliably found to not worsen the epileptiform activity, additional stimulation parameters were assessed including patterned stimulation (Wischnewski and Schutter, 2015) (Fig. 1). Different stimulation parameters were administered only after an interval of at least 15 min 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 were 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. The anesthesia dose was set to the absence of response to pinch of the hindleg skin. Anesthetic levels were kept constant throughout the experiment. 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 2 × 2 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 min. Epileptiform potentials developed in all