Selectivity of neural stimulation in the auditory system: a comparison of optic and electric stimuli

Agnella D. Izzo
Northwestern University
Biomedical Engineering
2145 Sheridan Road, Tech E310
Evanston, Illinois 60208

Eul Suh
Jyoti Pathria
Northwestern University
Feinberg School of Medicine
Department of Otolaryngology – Head and Neck Surgery
Chicago, Illinois 60611

Joseph T. Walsh, Jr.
Northwestern University
Biomedical Engineering
2145 Sheridan Road, Tech E310
Evanston, Illinois 60208

Donna S. Whitlon
Northwestern University
Feinberg School of Medicine
Department of Otolaryngology – Head and Neck Surgery
Chicago, Illinois 60611
and
Northwestern University
Institute for Neuroscience
Evanston, Illinois 60208-3520

Claus-Peter Richter
Northwestern University
Feinberg School of Medicine
Department of Otolaryngology – Head and Neck Surgery
Chicago, Illinois 60611

Abstract. Pulsed, mid-infrared lasers were recently investigated as a method to stimulate neural activity. There are significant benefits of optically stimulating nerves over electrically stimulating, in particular the application of more spatially confined neural stimulation. We report results from experiments in which the gerbil auditory system was stimulated by optical radiation, acoustic tones, or electric current. Immunohistochemical staining for the protein c-FOS revealed the spread of excitation. We demonstrate a spatially selective activation of neurons using a laser; only neurons in the direct optical path are stimulated. This pattern of c-FOS labeling is in contrast to that after electrical stimulation. Electrical stimulation leads to a large, more spatially extended population of labeled, activated neurons. In the auditory system, optical stimulation of nerves could have a significant impact on the performance of cochlear implants, which can be limited by the electric current spread. [DOI: 10.1117/1.2714296]

Keywords: optical stimulation; cochlear implant; current spread; c-FOS; laser; spiral ganglion cell.

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1 Introduction

Despite its shortcomings, electric current has, for centuries, been the gold standard for artificial stimulation of neural function.[1–4] However, complications can arise when the spread of current in the tissue results in activation of large neuron populations and tissue damage can occur from electrochemical changes at the electrode-tissue interface or from the electrode insertion. In addition, stimulation artifacts often plague experimental studies.

A novel method for neural stimulation can overcome many of the shortcomings related to electrical stimulation. Wells et al. showed that pulsed, IR optical radiation can evoke action potentials from the rat sciatic nerve.[5] The spatial resolution of stimulation is the foremost advantage of optical radiation over electrical current. Only tissue that is directly in the optical path absorbs the light. Furthermore, there is minimal light scattering in tissue when irradiated with mid-IR wavelengths.[6,7] Moreover, with optical radiation, it is possible to stimulate neural tissue without direct physical contact of the stimulator. Our work extended the studies of Wells et al. and demonstrated that a pulsed, IR laser will stimulate the gerbil auditory nerve.[8] With the assumption that light has less spread of excitation than electric current, optical radiation could be an innovative technology with great benefit for cochlear implants.

In individuals who are profoundly deaf, multiple-electrode cochlear implants are designed to directly electrically stimulate discrete spiral ganglion cell populations along the co-

Address all correspondence to Claus-Peter Richter, 303 East Chicago Avenue, Searle 12-561, Chicago, Illinois 60611; Tel: 312–503–1603; Fax: 312–503–1616; E-mail: cri529@northwestern.edu

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chlea, attempting to restore the tonotopic responses of the normal acoustically stimulated cochlea. Tonotopicity is the spatial gradient of response in the mammalian cochlea, in which high-frequency tones activate spiral ganglion neurons in the base of the cochlea and low frequencies activate neurons in the apex of the organ.[9–15] A successful multichannel cochlear implant should, therefore, transfer a maximum of information to discrete, spatially selected groups of auditory neurons. Stimulation at one electrode should not affect the neural response to stimulation resulting from neighboring electrodes.

However, the assumption that discrete neural populations can be electrically activated is not always true. Although it is widely assumed that stimuli applied between closely spaced bipolar electrodes can locally stimulate spiral ganglion cells, whereas widely spaced electrode pairs will lead to broad electric fields and will result in wide areas of neural activation,[16,17] it has been shown that closely spaced electrode pairs at high current levels will activate a broad region of auditory neurons.[16,18] If two electrodes stimulate the same neural population, sound sensation encoded via these two electrode contacts might be confused or even be indistinguishable and this will reduce the number of independent channels of information that can be conveyed to the cochlear implant user. This limitation is based on fundamental physical principles of electrical stimulation that even the best electrode design has not yet overcome.

In this paper, we demonstrate that optical energy can provide a more spatially selective stimulation of the auditory system than can electric current. To do so, we employed an immunohistochemical staining method for the protein product, c-FOS (for a review of c-fos regulation and expression, see Refs. 19 and 20). Here we compare the spatial extent of c-FOS expression in the spiral ganglion, after eliciting a compound action potential (CAP) in the eighth nerve by acoustic stimulation, laser pulses, or electrical current.

2 Materials and Methods

All measurements were made *in vivo* using adult gerbils (*Meriones unguiculatus*). The care and use of the animals in this study were carried out in accordance with the National Institutes of Health (NIH) *Guide for the Care and Use of Laboratory Animals* and was approved by the Animal Care and Use Committee of Northwestern University.

2.1 *Animal Surgery and Preparation*

Animal surgery was made as described previously.[21] Gerbils were anesthetized by an initial intraperitoneal injection of sodium pentobarbital (80 mg/kg body weight). Maintenance doses were 17 mg/kg body weight and were given throughout an experiment whenever the animal showed signs of increasing arousal, which was assessed every 30 min by a paw withdrawal reflex. After the animal was fully anesthetized, breathing was facilitated by performing a tracheotomy and securing a length of PE90 tubing into the opening in the trachea. The animal was then positioned, belly up, on a heating pad used to maintain body temperature at 38 °C, and its head was stabilized in a heated head holder. A dermal incision was made from the lower right jaw to the right shoulder to expose the right submandibular gland, which was subsequently ligated and removed. The muscles attached to the bulla and to the styloid bone were carefully dissected. Next, the bulla was opened to enable access to the cochlea. A silver electrode was hooked onto the bony rim of the round window of the cochlea, and a ground electrode was placed under the skin at the left jaw. After cutting the cartilaginous outer ear canal, a speculum (to connect the sound delivery system) was cemented with dental acrylic to the bony part of the outer ear canal. The surgical platform containing the animal was then moved onto a vibration isolation table in a soundproof booth. Two chest electrodes were attached to monitor heart rate, and a high-frequency tweeter (Beyer 770Pro) was coupled to the speculum at the ear canal.

2.2 *c-FOS Stimulation Experiments*

Following surgery, animals remained in a quiet environment for at least 1 h by placing them in a soundproof booth. This time period allowed for c-FOS expression to be reduced to a minimum prior to the start of stimulation. Following this quiet period, each animal was stimulated for 90 min with acoustic, laser, or current pulses. [See Izzo et al.[22] for input-output curves relating stimulus levels to evoked responses.]

2.2.1 *Optical stimulation*

The optical source was a Holmium:YAG laser (Laser 1-2-3, SEO; Orlando, Florida), with a wavelength of 2.12 μm and a pulse duration of 350 μs, operating at 2 Hz. The laser output was coupled to a low-OH 100-μm-diam optical fiber (FIP series, Polymicro; Phoenix, Arizona). The fiber was heated to 36 °C with a heating wire coil (NI60, Omega; Stamford, Connecticut) to prevent hearing loss on cooling the cochlea. The optical fiber was inserted at the basal turn of the cochlea, approximated to, but not penetrating, the round window membrane, and visually oriented toward the spiral ganglion cells, as enabled by the surgical access. The fiber was fixed in place and was not in direct contact with cochlear structures (Fig. 1). The radiant exposure was controlled by the number of heat-absorbing glass slabs placed in the beam path. A radiant exposure of 60 mJ/cm^2 was used to optically stimulate the gerbils. (Threshold for optically stimulated CAP in the gerbil cochlea is approximately 0.01 J/cm^2, Ref. 8.)

2.2.2 *Electric stimulation*

Electric stimuli consisted of charge balanced biphasic pulses (0.5 ms each phase). The pulses were generated using custom written software and a digital-to-analog computer board (KPCI-3116, Keithley) and were used to control an ac/dc current calibrator (Model 2500, Valhalla Scientific, San Diego, California). The stimulating electrodes were inserted 3 to 4 mm through the round window into the basal turn of the cochlea and directly connected to the current calibrator. Current amplitudes of 100 μA were applied. (The threshold for electrically evoked auditory brainstem response, eABR, is typically[23] in the range of 30 to 40 μA.) Stimulation was done with one electrode placed in the scala tympani and a second electrode placed in the jaw (reference electrode, as described in the surgical procedures already presented).

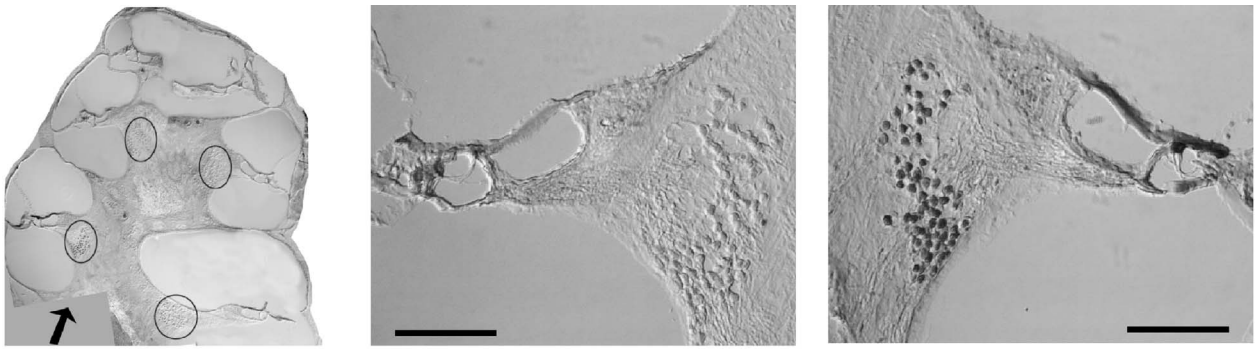

Fig. 1 Cochlear sections stained for c-FOS. (a) This midmodiolar tissue section from a gerbil cochlea indicates the images used to visualize the c-FOS-stained tissue. Locations of spiral ganglion cells are indicated by the black circles. The approximate placement and orientation of the optical fiber with respect to the cochlea is shown by the arrow. (b) A tissue section negatively stained for c-FOS, from the middle turn of the cochlea. Image captured at 20× with differential interference contrast (DIC). This cochlea was exposed to laser stimulation. The scale bar equals 50 μm. (c) A tissue section with spiral ganglion cells positively stained for c-FOS from the upper basal turn of a gerbil cochlea. This cochlea was exposed to laser stimulation. Image captured at 20× with DIC. The scale bar equals 50 μm.

2.2.3 *Acoustic stimulation*

As a control to verify c-FOS staining, a few animals were exposed to acoustic stimuli. The stimuli were 20-kHz tone pips at a 95 dB sound pressure level (SPL) delivered with a Dual L201 speaker in free field, placed 10 cm away from the unanesthetized animal. (The threshold for auditory CAP in the gerbil in response to tone pips at 20 kHz is approximately[24] 40 dB SPL). The tone pips were 15 ms long, with rise/fall times of 10 ms.

2.3 *Tissue Preparation*

After completion of the stimulation, the animals were euthanized with a 200 mg/kg-body-weight overdose of sodium pentobarbital. Each animal was decapitated and the cochleas were removed and placed in 4% paraformaldehyde in 0.1-M phosphate-buffered saline (PBS), pH 7.4. After 2 h, the cochleas were rinsed in PBS and placed for 2 weeks in PBS containing 10% ethylenediamine tetraacedic acid (EDTA), pH 7.4, at 4°C to decalcify. PBS was made from solutions of 0.2-M NaH_2PO_4 and 0.2-M Na_2HPO_4, to which 0.9% NaCl was added.

The cochleas were then prepared for sectioning on a cryostat by sucrose-embedding the tissue. For a detailed procedure of tissue embedding, see Ref. 25. In short, the tissue was incubated in increasing concentrations of sucrose solution for 30 min at each step, while on a tissue rotator at room temperature (10% sucrose; 2:1 solution of 10%:30% sucrose; 1:1 solution of 10%:30% sucrose; 1:2 solution of 10%:30% sucrose; 30% sucrose). Finally, specimens were stored overnight in 30% sucrose solution at 4°C. The next day, the cochleas were placed in degassed optimal temperature cutting compound (OCT, Tissue-Tek) and stored in OCT overnight at 4°C. The cochleas were frozen in OCT, using an ethanol and dry ice bath. Samples were stored at a temperature of −80°C until ready for sectioning.

The specimens were placed in the cryostat and allowed to equilibrate to the temperature of the cryostat (−20°C). The cochleas were oriented such that they were sectioned parallel to the modiolus [see Fig. 1(a) for midmodiolar section]. The tissue specimens were cut at 20 μm. The sections were mounted on biobond-coated superfrost microscope slide. After sectioning, the slides were allowed to air dry for 2 h. The edge of each slide was outlined with a hydrophobic PAP pen (Accurate Chemical, Westbury, New York) to prevent run-off of staining solutions. Then, the slides were stored in a freezer at −80°C until further use.

2.4 *Immunohistochemical Staining*

For staining, selected cochlear sections were removed from the freezer and were allowed to dry for 2 h. To further enhance tissue adhesion to the slide, the slides were placed in a vacuum for 15 min. The sections were then placed in 1.5% paraformaldehyde in 0.1-M $NaPO_4$ buffer, pH 7.2, for 5 min. The slides were washed in 0.05% Tween-20 in 0.05-M tris-buffered saline (TTBS), pH 7.6, for 2×5 min. Next, slides were placed in a peroxide buffer (containing 8 ml of 30% H_2O_2, 20 ml MeOH, 72 ml distilled H_2O, and 100 ml TBS) for 10 min and, again, washed in TTBS for 5 min. Slides were then covered with 300 μl/slide of blocking solution, composed of 10% normal donkey serum (017-000-121, Jackson Immunoresearch), 10% non-fat dry milk, 80% TBS. Then, slides were incubated at room temperature for 2 h. After 2 h, the blocking agent was removed and the primary antibody (Rabbit Anti c-FOS F7799, Sigma-Aldrich; 1:8000 dilution in 10% non-fat dry milk, 10% donkey serum, and TBS to final volume) was applied (300 μl/slide). The slides were incubated at 4°C overnight. The following day, the slides were washed five times with TTBS for 5 min each. The secondary antibody [Donkey Anti-Rabbit IgG (immunoglobulin G) 711-036-152, Jackson Immunoresearch; 1:100 dilution in 10% non-fat dry milk and TBS to final volume), 300 μl/slide, was then applied and incubated for 4 h at room temperature. Slides again were washed twice in TBS alone, 5 min each. Next, the edges of the slides were blotted dry and the sections treated with diaminobenzidine (DAB kit SK-4100, Vector Laboratories), 200 μl/slide. The staining was stopped by washing the slides three times in TBS, 5 min each.

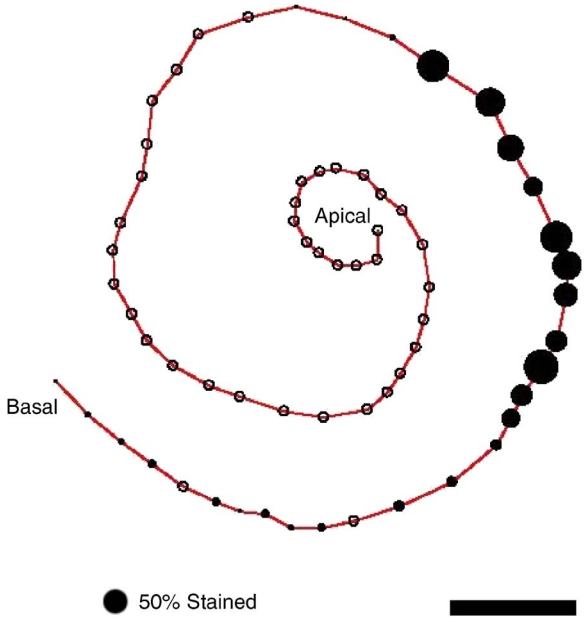

Fig. 2 c-FOS stimulation map from acoustically stimulated cochlea. c-FOS cell counts were plotted on a reference map corresponding to the known anatomical location of spiral ganglion cells in the gerbil cochlea. The filled black circles indicate where c-FOS staining was observed in the cochlea, with the size of the circle indicating the percent of stimulated cells. Unfilled circles indicate where there was no c-FOS staining evident in the tissue section. All c-FOS data presented in this paper follow the same scaling of data markers. This is a representative cochlea that was exposed to 20-kHz tone pips at 95 dB SPL. Some c-FOS staining was evident in the base, but the majority of the c-FOS staining occurred in the upper base. The scale bar equals 500 μm.

Slides were air dried, mounted with Permount, and covered with a cover slip.

Images of each tissue section were taken on a Zeiss microscope (Axio Imager A.1) by bright-field illumination and DIC at 2.5×, 20×, and 40×. The number of stained and non-stained spiral ganglion nuclear profiles at each turn of the cochlea were counted on each tissue section [Fig. 1(a)], and a percentage of stained cells was calculated.

To assist with the data analysis of c-FOS staining, we reconstructed the center line of the gerbil spiral ganglion. For the reconstruction, a gerbil cochlea was harvested and processed by the procedure already described, with the difference being that the cochlea was sectioned at 20 μm perpendicular to the modiolus, rather than parallel to the modiolus. The resultant sections were imaged for every section at 2.5× magnification. The images were coaligned with respect to each other by anatomical references. The location of the spiral ganglion cells in each section was determined and the distances from the spiral ganglion cells to a fiducial marker were measured. These distances resulted in an x and y coordinate for each group of spiral ganglion cells. The depth of the cochlear section gave the z coordinate. These coordinates were then input into a 3-D matrix in IgorPro. The percentage of stained cells for each stimulated cochlea were then plotted on this standardized 3-D reference map of spiral ganglion cell locations. The size of the data markers on the reference maps corresponds to the percentage of stained cells, with a larger marker indicating a larger percent stained. The markers are presented on the same scale across all spiral ganglion cell maps.

3 Results

In response to acoustic, optic, and electric stimuli, gerbil spiral ganglion cells expressed c-FOS. An example of a tissue section stained for c-FOS is shown in Fig. 1(c). The darkened spiral ganglion cells are evident in this image. In contrast, unstained spiral ganglion cells from the same cochlea are shown in Fig. 1(b). Control experiments, in which the antibody was preadsorbed with the immunizing peptide, showed no c-FOS staining (data not shown). A total of 68 cochleas were processed by this method in our laboratory, 15 of which were stimulated with the correct stimulus level and processed with the correct c-FOS antibody concentrations (4 electric, 5 optic, 6 acoustic).

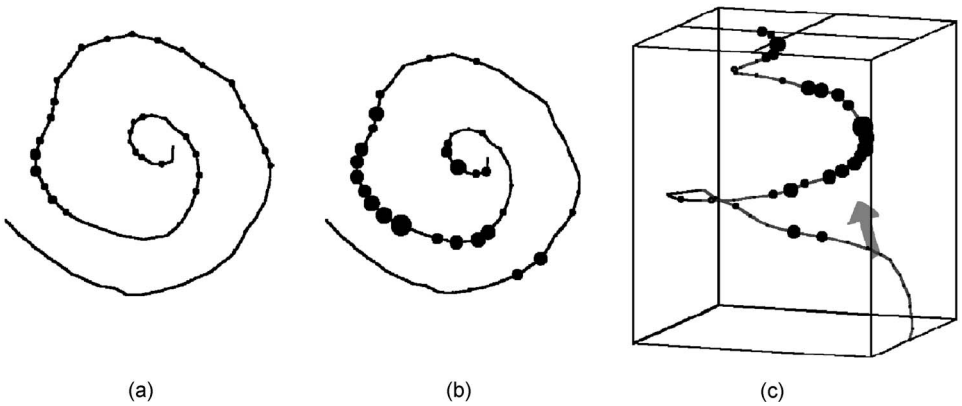

Fig. 3 Optically stimulated cochleas reveal selective c-FOS staining. c-FOS staining of representative optically stimulated cochleas indicates that the only cells stimulated were those directly in the optical path. (a) and (b) When the optical fiber is inserted through the round window and directed parallel to the modiolus, the optical path interacts with the upper base and upper middle turns. The amount of staining in the upper base was larger than in the upper middle. There was no staining evident at other turns in the cochlea. (c) Note the orientation of the optical fiber as shown by the gray arrow. This is a 3-D side view of the same cochlea shown in (b). Each cochlea was stimulated with 0.06 J/cm^2.

To better compare acoustic, optic, and electric stimulation used in this experiment, we mapped all of the analyzed data to the reconstructed gerbil spiral ganglion map (Fig. 2). The size of the filled circles in the map represents the percentage of labeled cells in that region of the cochlea. The activation pattern shown in Fig. 2 resulted from a cochlea acoustically stimulated with 20-kHz tone pips. In the basal portion of the cochlea, only a few spiral ganglion cells were stained for c-FOS, while the highest amount of staining occurred in the upper basal portion, with ~60% of the cells stained. More apically from the large peak in c-FOS expression in the upper base, we observed several data points with 5 to 10% of cells stained for c-FOS expression. No c-FOS staining was visualized elsewhere in the cochlea.

We obtained positive c-FOS staining in response to laser stimulation of the cochlea (Fig. 3). After laser stimulation, c-FOS was expressed primarily in the tissue that was directly in the optical path of the laser beam. The fiber orientation for the cochleae in Figs. 3(a) and 3(b) is shown in Fig. 3(c). The largest fraction of c-FOS staining was observed in the upper basal portion of the cochleas, with a little staining observed in the upper middle portion of the cochleas. In the cochlea shown in Fig. 3(a), the maximum amount of cells stained in the upper base was 40%; in the cochlea in Fig. 3(b), the maximum amount of cells stained was 79%. When the orientation of the optical fiber was changed such that the fiber was oriented at a shallow angle to the modiolus (rather than parallel to the modiolus as in previous examples), we observed a different pattern of staining; the majority of the staining occurred in the base of the cochlea, directly in the optical path, and there was some staining in the middle of the cochlea. The maximum amount of staining seen was 46% (Fig. 4).

In the electrically stimulated cochleas, spiral ganglion cells stained for c-FOS were observed in all turns of the cochlea, throughout every tissue section examined (Fig. 5). There was no obvious spatial confinement of the stimulated cells, as in the optically stimulated cochleas. There was some variability of the percentage of stained cells within each cochlea. In the first cochlea shown in Fig. 5(a), the average percent of cells stained was 67±8% [mean ± SE (standard error), range 51 to 80%, 30 spiral ganglion locations visualized]. For the cochlea shown in Fig. 5(b), the average percent of cells stained was 42±18% (range 10 to 100%, 54 spiral ganglion locations). The third cochlea pictured in Fig. 5(c) had 44±12% of cells stained (range 14 to 67%, 58 spiral ganglion locations). In Fig. 5(b), a maximum of staining is observed in the middle portion of the cochlea, with local maxima also observed one full turn more basal and more apical in the spiral ganglion.

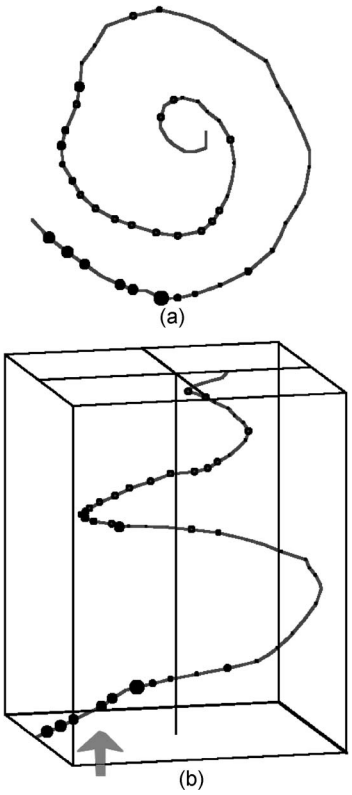

Fig. 4 Changing orientation of optical fiber changes stimulated population. (a) When the orientation of the optical fiber was changed such that it was irradiating at a shallow angle to the modiolus, the positively stained spiral ganglion cells occur mostly in the basal portion of the cochlea and some in the middle of the cochlea. (b) Note the orientation of the optical fiber as shown by the gray arrow. This is a 3-D side view of the same cochlea shown in (a). The cochlea was stimulated with 0.06 J/cm^2.

4 Discussion

We demonstrated that spiral ganglion cells in the gerbil cochlea express c-FOS in response to optic, electric, and acoustic stimuli, using an immunohistochemical staining method. Our data show that laser stimulation of the cochlea can provide a more spatially selective stimulation than electric current. Optical energy does not spread or scatter in the tissue. Rather, at mid-IR wavelengths, the light is absorbed by the volume of tissue/fluids directly in the optical path. This is evident in the staining results, as neural activation occurred directly opposite where the optical fiber was placed. It is clear that no optical energy was delivered to the contralateral side of the cochlea. In contrast, electrically stimulated cochleas did not demonstrate a spatially confined c-FOS staining. The results demonstrated the spread of electric current that is injected into the cochlea.

Note that the optical energy was not totally absorbed within the basal turn of the cochlea and that staining was also observed in the middle turn. This is to be expected based on the wavelength selected for this study. The distance from the tip of the optical fiber to the stained spiral ganglion cells in the middle turn of the cochlea is ~650 μm. At 2.1 μm, the optical penetration depth in water is ~450 μm, which describes the distance over which the incident energy is reduced by 66%. It is easy to adjust the optical penetration depth (OPD) by varying the wavelength of the light. Therefore, it would be possible to stimulate only the spiral ganglion cells in the basal turn by matching the OPD to the distance to the target tissue. We have conducted experiments to examine the effect of wavelength on optical stimulation in the gerbil cochlea.[26]

Note also the staining in the acoustically stimulated cochlea. There was a large maximum in c-FOS staining in the upper base of the cochlea. In addition, we saw a very small amount of staining (5 to 10%) in three data points more apical

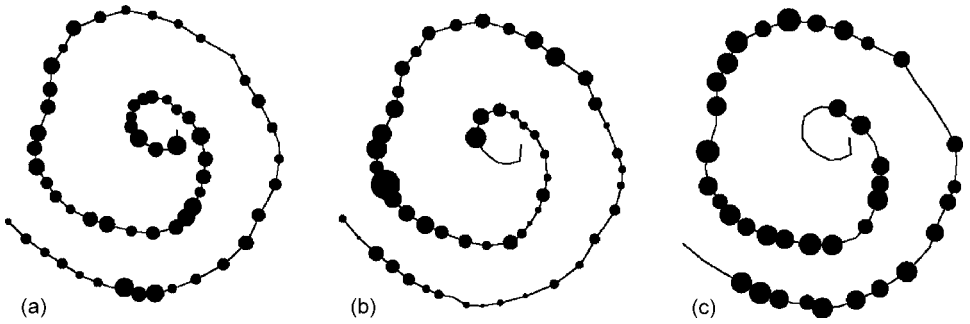

Fig. 5 Electrically stimulated cochleas exhibit broad c-FOS staining. Representative cochleas that were electrically stimulated reveal c-FOS staining throughout every tissue section across every turn. (b) In this electrically stimulated cochlea, a local maximum of stimulation is seen in the upper middle turn, with a significant amount of staining more basally from this maximum as well as in the apex. (c) A local maximum of c-FOS staining is seen in the base of the cochlea as well as one full turn higher. However, there seems to be a spread of neural excitation from the middle of the cochlea toward both the apical and basal directions. All cochleas were stimulated with 0.5-ms biphasic pulses at 100 μA. The stimulating electrode was inserted 3 to 4 mm into the basal turn of the cochlea.

from the maximum. One possible explanation for this is that it represents the activation of type II spiral ganglion cells. Type II spiral ganglion cells comprise $\sim$5% of the spiral ganglion cells in the cochlea and are afferent innervations of the outer hair cells. The projections of type II cells extend slightly more apical from their entry to the organ of Corti.[27,28]

Note that *c-fos* is an early immediate gene that is expressed in neuronal cells in response to membrane electrical signals.[29] Typically, in neural cells, it is undetectable until the cell receives a stimulus, at which point *c-fos* is upregulated within minutes. Immunohistochemical staining of the protein product, c-FOS, has been demonstrated in the auditory system of rodents in response to acoustic stimuli[30–32] as well as electric stimuli.[33–36] However, this c-FOS-staining method has some limitations. At low to moderate levels of stimulation for all three types of stimuli, no spiral ganglion cells were stained for c-FOS (the shortcomings of the c-FOS method are discussed in greater detail in Izzo et al.[22]). To achieve c-FOS staining, stimulation levels needed to be well above threshold levels to elicit a CAP. In other words, the present data reflect high-level cochlear stimulation. This holds for acoustical, electrical, and optical stimulation. Consequently, relatively large populations of spiral ganglion cells are stimulated and express c-FOS. To our knowledge, only two other groups have published results on c-FOS immunohistochemical staining for neural structures in the cochlea. Shizuki et al. documented c-FOS expression in the guinea pig cochlea in response to noise exposure.[37] Saito et al. reported the cochlear c-FOS expression following electrical stimulation in the cochlea.[35] They, too, were only able to achieve positive c-FOS staining in all electrically stimulated cochleas when using high current levels. At the high level of stimulation, Saito et al.[35] reported a wide spatial distribution of c-FOS-stained cells.

The results of our experiments clearly demonstrate that optical energy can selectively stimulate neural tissue. Although mechanisms for high-power laser-tissue interactions have been thoroughly characterized and described,[6,7] the mechanism by which low-power laser-tissue interactions occur remains equivocal. After conducting several control studies, Wells et al. concluded that the most likely mechanism of laser stimulation of nerves is a photothermal effect.[38] At mid-IR wavelengths, there is little light scattering in tissue and the primary method of light-tissue interaction is the absorption of the light by water in the tissue. On absorption, this optical energy is transferred to thermal energy and results in heating of the target area. Subsequent to the local transient temperature increase, ion flux may occur through either nonspecific holes formed by poration of the cell membrane or by activation of ion channels. Future experiments of optical stimulation in the gerbil cochlea include investigating the mechanism of stimulation.

The results of this study (1) verify that optical energy does not spread in the tissue as electric current does and (2) raises the possibility that future neural prostheses can be built using optical energy as the stimulation source to achieve a better resolution of neural stimulation. By substituting an optical source for electrodes in cochlear implants, it may be possible to confine neural activation to a spatial area immediately adjacent to the optical source. Spatial confinement of neural activation could lead to improved performance by implant users.

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