

First Quarterly Progress Report

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Neurophysiology Studies of Stimulated Auditory
Prostheses

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Introduction

Neural prosthetic devices are artificial extensions to the body that restore or supplement nervous system function that was lost during disease or injury. Particular success has been realized in cochlear prostheses development. The devices bypass damaged hair cells in the auditory system by direct electrical stimulation of the auditory nerve. Stimulating discrete spiral ganglion cell populations in cochlear implant users' ears is similar to the encoding of small acoustic frequency bands in a normal-hearing person's ear. In contemporary cochlear implants, however, the injected electric current is spread widely along the scala tympani and across turns. Consequently, stimulation of spatially discrete spiral ganglion cell populations is difficult. One goal of implant device development is to design cochlear implants that stimulate smaller populations of spiral ganglion cells. In contrast to electrical stimulation, extreme spatially selective stimulation is possible using light.¹⁻⁵ Therefore, the goal is to develop and build optical cochlear implant prostheses to stimulate small populations of spiral ganglion cells. Steps towards this objective include (1) quantify the optical parameters that allow for safe spiral ganglion cell stimulation over extended periods of time, (2) characterize the fundamental spatial and temporal properties of optical stimulation of the auditory nerve, (3) determine the spatial resolution for laser stimulation. By accomplishing the first three goals within the first three years, we will be able (4) to build and implant the first animal cochlear implant electrode for long-term safety studies during years four and five. Also, the results will provide a basic set of parameters that can be used for other neural interfaces that use optical radiation to stimulate neurons.

During the last quarter we worked on Step I: Quantify the parameters that allow the safe use of laser radiation for auditory nerve stimulation. The objectives for Step I are: Acute experiments are made in normal hearing and in long-term deafened gerbils. An optical fiber is placed after surgical access to the cochlea close to the modiolus. The optical fiber is coupled to an optical radiation source. While the auditory system is stimulated with light pulses, compound action potentials are recorded at the round window for different stimulus parameters: radiation wavelength, pulse length, pulse repetition rate, increasing optical energy, extended stimulation times, different diameters of the optical fiber between 50 and 600 μm , variable fiber distances and orientations from the spiral ganglion cells in the modiolus, and for different locations of optical fiber placement along the cochlea. Measuring the peak-to-peak amplitude of the optically evoked potential serves to monitor cochlear function. The CAP amplitude decreases when cochlear damage occurs. The results provide the safe stimulation parameters for optical stimulation of the auditory system.

Summary of activities from September 1, 2006 to January 31, 2007:

Publications resulting from the activities (copies of the manuscripts are enclosed and posters will be accessible on the internet after February 16, 2007)

1. Izzo, A.D., M. Bendett, M.E. Jansen, E., Jim Webb, Heather Ralph, Walsh, Jr., J.T., Richter, C.-P. (2007) Optical Parameter Variability in Laser Nerve Stimulation: a study of pulse duration, repetition rate, and wavelength. IEEE Transactions on Biomedical Engineering (in press).
2. Izzo A.D., Littlefield, P., Walsh, Jr., J.T., Webb, J., Ralph, H., Bendett, M., Jansend, D.E. and Richter, C.-P. (2007) Laser stimulation of auditory neurons at high repetition rate, SPIE (in press).
3. Suh, E., Agnella D. Izzo, A.D., Walsh Jr., J.T., Richter, C.-P. (2007) The role of Transient Receptor Potential channels in neural activation. Abstr. Assoc. Res. Otolaryngol. 30, 109.
4. Izzo, A.D. Lin, A., Oberoi, M., Walsh, Jr.¹, J.T., and Richter, C.-P. (2007) Tone-on-light masking reveals spatial selectivity of optical stimulation in the gerbil cochlea. Abstr. Assoc. Res. Otolaryngol. 30, 446.
5. Littlefield , L., Izzo, A.D, Mundi, J., Walsh, Jr., J.T., Jansen, D.E., Bendett, M., Webb, J. Ralph, H., Richter, C.-P. (2007) Laser stimulation of the auditory nerve stimulation is possible at high repetition rates. Abstr. Assoc. Res. Otolaryngol. 30, 356.

Personnel involved

Paid by the grant

Izzo, A.: post-doctoral student
Otting, M.: Technician
Richter, C.-P.: PI

Part of their training

Littlefield, P.: Neurootology Fellow
Teudt, I.: Post-doctoral student from Hamburg (Germany)
Mundi, J.: Medical student
Lin, A.: Medical student
Suh, E.: Pre-medical student
Nevel, A.: Pre-medical student
Bayron, R.: Resident of the Department of Otolaryngology

Technical activities

We revised the offers for a multi-channel recording system. For further information and feedback, we contacted Dr. Shepherd who was very helpful in sharing his expertise and results he had during the acquisition of such a recording system. We placed the order for a multi-channel recording system from Plexon in January 2007.

In December we were able to hire a technician, Margarete Otting, to support the project.

We generated a Website that is linked to the PI's name on the Departmental Website: <http://www.oto-hns.northwestern.edu/Richter%20Lab/index.htm>. The Website will be updated regularly.

Scientific activities

Summary

Research activities on the project have already been started before the contract has been awarded. From those activities data were already available. The publication of these data was finalized. Furthermore, single fiber experiments were continued, which were also started before the contract has been awarded. The data are not complete and experiments will continue during the next quarter. Animals have been deafened to validate the laser parameter in longterm deafened animals.

Compound action potential (CAP) measurements in gerbils

Measuring the peak-to-peak amplitude of the optically evoked compound action potential (CAP) from the cochlea serves to monitor cochlear function. The CAP amplitude decreases when cochlear damage occurs. Compound action potentials were recorded at the round window for different stimulus parameters:

- radiation wavelength (within a limited range),
- pulse length,
- pulse repetition rate,
- increasing optical energy,
- extended stimulation times.

Results show that pulse durations as short as 35 μ s elicit compound action potentials from the cochlea. In addition, repetition rates up to 400 Hz can continually stimulate cochlear spiral ganglion cells for extended periods of time. Varying the wavelength, and therefore the optical penetration depth, allowed different populations of neurons to be stimulated. The results were obtained from normal hearing animals and are detailed in the manuscript attached to this report. Moreover experiments have been started to verify the results in acutely deafened and in longterm deafened animals. To this point 10 acutely deafened animals have been examined. Data analysis is still underway and will be finished within the next quarter.

Single fiber measurements in gerbils

Activities of single auditory nerve fibers were recorded, while the cochlea was stimulated with optical radiation. At present, we recorded from 150 single auditory nerve fibers, which responded to acoustic stimuli. After determining the characteristic frequency and the threshold of the fiber, the cochlea was stimulated with optical radiation using an optical fiber that was coupled to the Aculight Renoir Laser ($\lambda=1.844-1.873\mu\text{m}$). The fiber was placed in front of the round window, so that the light beam was directed towards the spiral ganglion cells. Data were collected from 35 of the auditory nerve fibers while irradiated. The following parameters were varied while the activity of the nerve fiber was recorded for 10s time intervals:

- increasing optical energy,
- pulse length,
- pulse repetition rate,

Increasing the radiant exposure leads to changes in firing patterns of an auditory nerve fiber (Fig. 1). Action potentials (APs) occur in a random pattern when no stimulus is presented to the ear. After the radiant exposure reaches a threshold value, APs occur directly after the optical pulse (Fig. 1). Further increase in radiant exposure resulted in pairs of APs (Fig. 1, top traces). The maximum repetition rate for the optical pulses was 13 Hz for the initial experiments. The shortest time delay between the optical pulse and the first action potential was 2.5 ms, which would correspond to a rate of 400 Hz.

In subsequent experiments, we tested the effect of increased repetition rates of the optical pulses on the rate of APs. The rate of the optical pulses was varied between 100 and 1000Hz. An example is shown in Figure 2. While the action potentials occur strictly after the optical pulse for optical pulse repetition rates below 300 Hz, it is not the case for higher stimulus repetition rates. We calculated the autocorrelation function of the nerve fiber recordings to quantify the effect of stimulus repetition rate on pattern of APs. For low optical pulse repetition rates, APs occur at defined time intervals that correspond to the time intervals between the optical pulses (Fig. 3, bottom traces). Although the overall rate of APs is increased, the APs occur at random phase relative to the stimulus (Fig. 3, top traces).

Pulse width of the optical pulses is important for the generation of action potentials. The two examples in Figure 4 show that the increase in AP rate with increasing radiant energy differs for different pulse length. In particular, if the pulses becoming shorter, less energy is required to increase the rate (Fig. 4).

Changes in wavelength also affected the increase in AP rate with increasing radiant energy.

Tone-on- light masking

The experiments have been started before the award has been made and have been continued during the first quarter. They will answer some of the questions of Stage I. The experiments determine the spatial selectivity of stimulation with optical radiation, here in the gerbil. The experiments will be verified in a different animal model, the guinea pig, as soon as the multi-channel recording system is in place.

For the present experiments, we have used a masking technique in the gerbil cochlea to determine the frequency selectivity of optical stimulation. CAP masking experiments were conducted with the probe response evoked by light, rather than the conventional probe tone. The placement of the optical fiber determined the probe frequency. A continuous masker tone was presented simultaneously to the light. In a similar manner to tone-on-tone masking, the masker level was determined such that it reduced the laser evoked CAP by 6 dB. From this method, we constructed tuning curves, which we term tone-on-light tuning curves, as a measure of the selectivity of laser stimulation in the cochlea. For comparison, tuning curves were also obtained for tone-on-tone masking and tone-on-electrical masking.

In the present experiments, a masking method was used to determine the spatial selectivity of optical stimulation. The resulting tuning curves are similar in bandwidth to tone-on-tone masking curves, indicating a spatially localized optical stimulation of the cochlea (Fig. 5a). We measured tone-on-light tuning curves with best frequencies (BF) between 6 - 11 kHz (n=9). Tone-on-tone tuning curves with similar best frequencies are shown on the graphs for comparison purposes. When the optical fiber was moved within the round window of the same animal, two tone-on-light masking curves of different BF were measured (Fig. 5b). This demonstrates that the placement of the optical fiber determines the "frequency" of stimulation in the cochlea.

To further quantify the selectivity of the optical stimulation, we calculated the Q_{10dB} ratio, which describes the sharpness of tuning. A larger Q_{10dB} value occurs when the notch in the tuning curve is deeper and narrower; that is when the stimulation is more localized. For most tone-on-light data, we measured the Q_{10dB} ratio to be around 2 - 3. For comparison, the Q_{10dB} ratio for tone-on-tone curves was between 1.5 - 4. A manuscript with a more detailed description of the results is in preparation.

Deafening of animals

Gerbils were deafened by infusing different concentrations (0.05 M and 0.1 M) of neomycin into the middle ear cavity. Cochlear function will be determined using acoustic and optical stimuli in four weeks, after neural degeneration has been established. Spiral ganglion cell counts will be correlated with spiral ganglion cell density.

Symbiotic activities

Facial nerve measurements

Tumors of the parotid or thyroid gland, as well as neoplasias close to the skull base, often require surgical resection. One detrimental consequence of the surgery is the iatrogenic damage of nerves within the surgical field. Nerve damage can be prevented, however, if the nerve is identified during the surgeries, frequently accomplished with the use of nerve stimulators. Contemporary devices use electric current to stimulate the nerve. Although such devices help to decrease the incidence of nerve damage during surgery, two main limitations still exist: (1) Nerve damage can occur due to the physical

contact of the handheld device applying the electric current and (2) the stimulation of the neural tissue is unselective because of the vast spread of the current through the tissue. The hypothesis is that the use of light (pulsed infrared optical radiation) as a stimulus can overcome these limitations and might be valuable as a new screening device.

To demonstrate that the facial nerve can be stimulated at its trunk safely and selectively, the gerbil facial nerve trunk was exposed to 250 μ s light pulses at repetition rates of 2 Hz with the resulting muscle action potentials recorded. After light radiation nerve samples were examined for possible tissue damage using light microscopy.

Eight facial nerve trunks were stimulated optically with energies between 0.71-1.77 J/cm², resulting in compound muscle action potentials (CmAPs) throughout the facial muscles. These CmAPs were simultaneously measured in three locations: the m. orbicularis oculi, m. levator nasolabialis, and m. orbicularis oris, with resulting CmAPs (peak to peak) of 0.3-2.3 mV, 0.15-1.4 mV and 0.3-2.3 mV, respectively, depending on the radial location of the optical fiber and the used laser energy. Nerve branches (mandibular, buccal, and zygomatic) were also stimulated optically, resulting in CmAPs between 0.2 and 1.6 mV. Histological examination of the samples with light microscopy revealed clear tissue damage at a radiation energy of 2.66 J/cm², but no damage at a radiation energy of 2.22 J/cm².

The experiments support the benefits of optical nerve stimulation outlined above. Selective muscle action potentials were optically elicited in the gerbil facial nerve without direct physical contact. A neurostimulator with a fine spatial resolution and minimal impact required to preserve the nerve function during nerve related surgery can be developed.

TRPV1 channel knockout mice

Lasers can be used to stimulate neural tissue such as the sciatic nerve or auditory neurons. Wells and coworkers suggested that neural tissue is likely stimulated by heat (SPIE, 2006). Potential ion channels that can be stimulated by heat are the Transient Receptor Potential Vanilloid (TRPV) channels, a subfamily of the Transient Receptor Potential (TRP) ion channels. TRPV channels are nonselective cation channels found in sensory neurons involved in nociception. These channels are activated by various chemical stimuli, particularly by vanilloid compounds such as capsaicin (the ingredient found in hot chili peppers) and resiniferatoxin. Furthermore, TRPV channels can also be thermally stimulated. The activation temperature for the different TRPV channels varies and is 43°C for TRPV1 and 39°C for TRPV3.

Previous studies have documented the presence of TRPV1 in the rat cochlea. Our study builds upon these findings to show that TRPV1 channels are also expressed in the spiral ganglion cells of the gerbil cochlea. By performing an immunohistochemical staining procedure on frozen 20 μ m cochlear slices using a primary TRPV1 antibody, we observed specific immunostaining of the spiral ganglion cells. In control experiments, the primary TRPV1 antibody was pre-adsorbed with synthetic blocking peptide, resulting in the absence of staining.

In case the TRPV1 channel is activated by optical radiation, an animal missing the TRPV1 channel should have normal hearing to acoustic stimulation

but significantly elevated thresholds for stimulation with optical radiation. This hypothesis was tested using a knockout mouse for the TRPV1 channel. While thresholds for acoustic stimulation were similar, thresholds for stimulation with optical radiation were significantly elevated. Results will be presented as a poster at ARO 2007.

Plan for the next quarter

1. Further analyze the data and prepare the single fiber data for publication
2. Analyze and summarize the data obtained in acutely and chronically deafened animals
3. Repeat the measurements for non-deafened animals in long term deafened animals
4. Implement and test the multi-channel system
 - a. for different locations of optical fiber placement along the cochlea.

Appendix

Figures

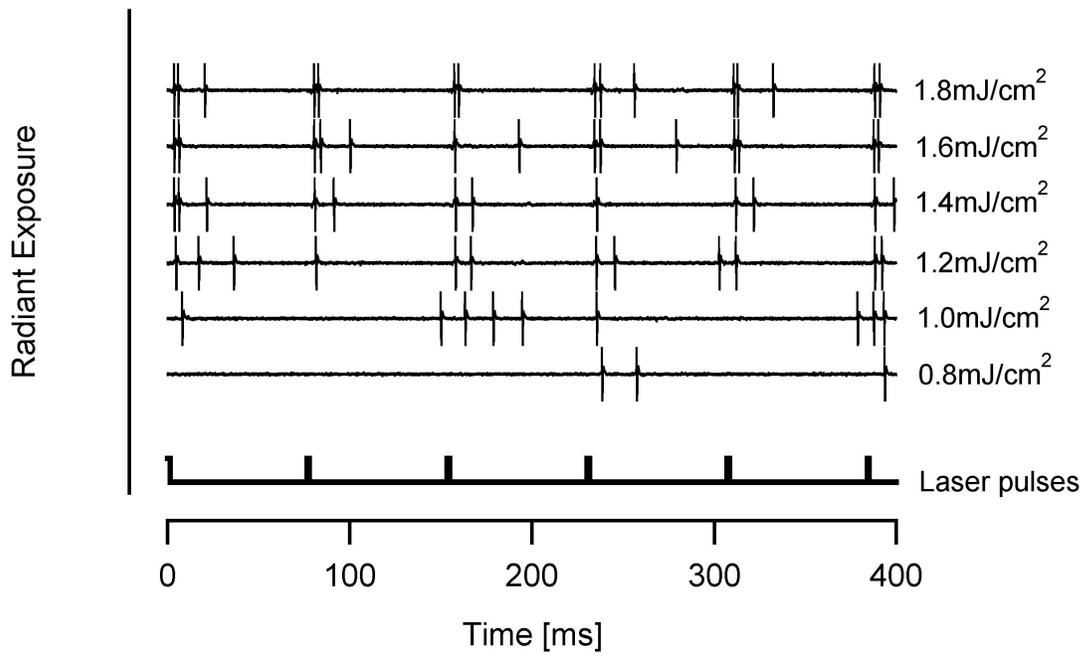


Figure 1: Recordings from a glass pipette in contact with an auditory nerve fiber. Action potentials are the spikes in the traces. Increasing radiant exposure results in an increase in rate. Furthermore, the increase in radiant exposure resulted in the occurrence of two subsequent action potentials (see upper trace). The lower trace shows the incidence of an optical pulse from the laser.

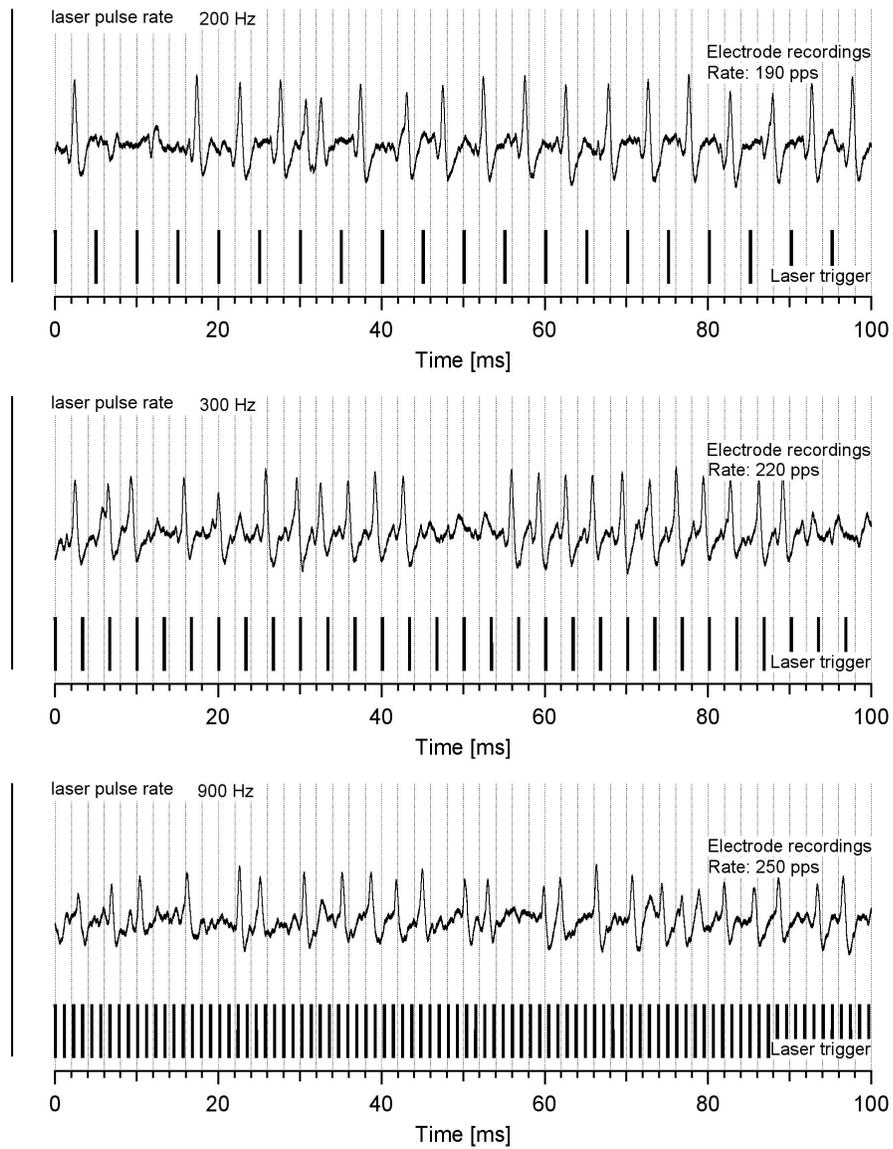


Figure 2: Recordings from a glass pipette in contact with an auditory nerve fiber. Action potentials (APs) are the spikes in the traces. Increasing the repetition rate of the optical pulses lead to an increase in number of APs per second. Here, the rate of APs did not increase above 250 APs/s.

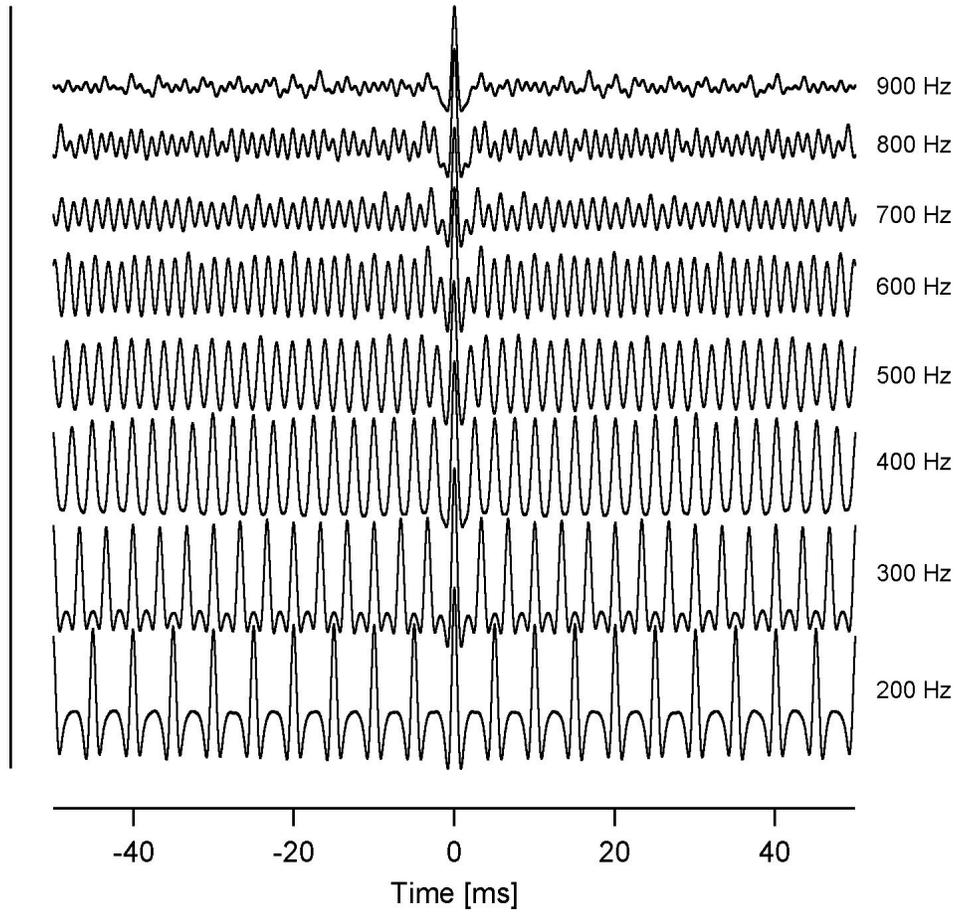


Figure 3: Shown is the autocorrelation calculated by using the recording from the neuron in Figure 2. While at 200 Hz optical stimulation rate, action potentials occur at time intervals that correlate with the stimulus repetition rate (see peaks in the autocorrelation function), at increasing stimulus frequencies, the action potentials occur more randomly.

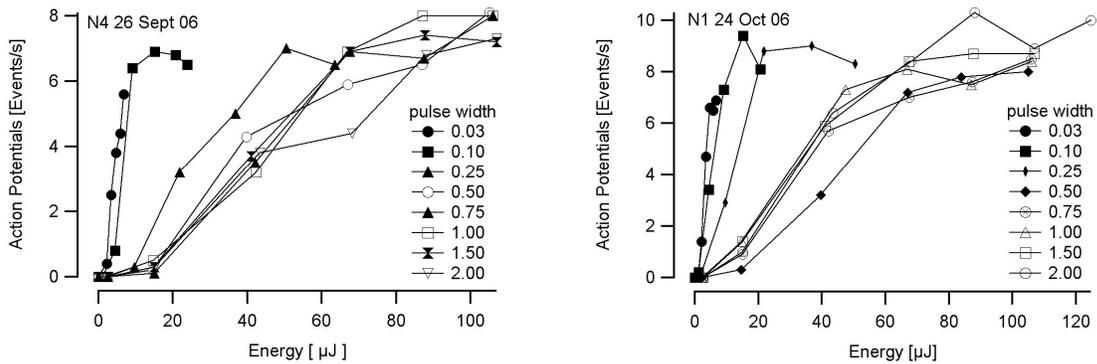


Figure 4: Shown are the rates of action potentials recorded from auditory neurons while optical stimulation occurred with different radiant energies. Traces represent different pulse widths of the optical radiation. The repetition rate of the optical pulses was 13 Hz.

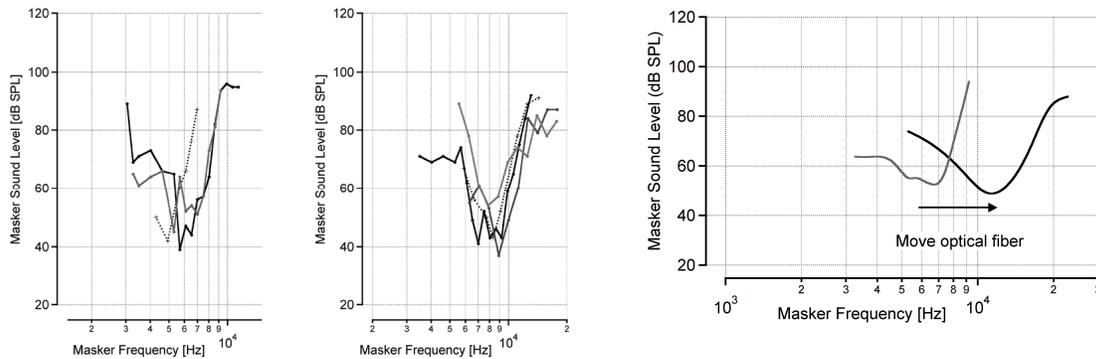


Figure 5a: Tone-on-light tuning curves demonstrate spatially selective optical stimulation. The tone-on-light masking curves, shown in solid lines, are similar in shape and extent to the tone-on-tone masking curves, shown in dotted lines. The data are presented on two separate graphs, grouped according to BF, for clarity of presentation. Each masking curve shows the raw data measured from a separate animal.

Figure 5b: The best frequency of tone-on-light tuning curves depends on optical fiber position. Two tone-on-light masking curves were recorded from the same animal, but with the optical fiber in a different position for each curve. The results show a corresponding shift in best frequencies from 6.7 to 10.8 kHz. These data are smoothed.