9th Quarterly Progress Report

October 1, 2005 through December 30, 2005

Neural Prosthesis Program Contract #N01-DC-3-1006

Protective and Plastic Effects of Patterned Electrical Stimulation on the Deafened Auditory System

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ABSTRACT

WORK COMPLETED DURING THIS QUARTER

1) Two neonatally deafened subjects continued chronic electrical stimulation with CII ™ processors (™ Advanced Bionics Corp) throughout the past quarter and during the next quarter will be studied in terminal acute electrophysiological studies recording from the inferior colliculus. Data collection will focus on spatial tuning curves (multichannel probe recording), forward masking (single- and 2-channel paradigms), modulation depth studies with sinusoidally amplitude modulated (SAM) pulse trains, and 2-channel interaction experiments.

2) Data analyses of spiral ganglion survival, and cochlear nuclear morphology along with electrophysiological data will continue in the new experimental group of subjects that have been deafened at 30 days rather than neonatally (including the new subject studied acutely last quarter). The goal of this series to examine possible critical periods in the anatomical effects of deafness and chronic electrical stimulation on the cochlea and cochlear nucleus.

3) A normal adult cat was studied in an acute electrophysiological experiment utilizing the 32-channel NeuroNexus probe. A brief acoustic calibration procedure was conducted during which a penetration site in the IC was selected and an ideal penetration depth determined based on the range of characteristic frequencies recorded, which must encompass the range of frequencies accessed by the cochlear implant electrode. Next, the animal was deafened by an injection of kanamycin followed by infusion of ethacrynic acid to effect, as indicated by elevation of ABR thresholds. Finally, an 8-channel UCSF electrode was implanted and responses to electrical stimuli were recorded. Data collection included electrical spatial tuning curves, masking protocols, SAM modulation depth and 2-channel interaction (as in the studies of chronic subjects in which the functional changes elicited by chronic stimulation (plasticity) are being evaluated.

4) Studies of the human cochlea were continued, and during this past quarter Dr. Leake presented some of the findings from this study at the First International Electro-Acoustic Workshop in Toulouse, France. A paper was submitted to the journal Audiology Neuro Otology for the workshop proceedings.

5) In addition, during the last quarter, we completed testing and fabrication of a new feline cochlear implant array that incorporates an osmotic pump a drug delivery system into an electrode array capable of delivering chronic electrical stimulation via 4 intracochlear electrodes. In a recent pilot study, one of these devices was successfully implanted in a 4 week-old kitten for intracochlear infusion of Brain Derived Neurotrophic Growth Factor (BDNF) combined with electrical stimulation of the cochlea. The device was maintained for 6 weeks with one change of the osmotic pump). The details of this new project are outlined in this QPR.

6) Four abstracts were submitted and accepted for poster presentations at the 2006 Association for Research in Otolaryngology Midwinter (appended at end of QPR).

INTRODUCTION

Several aspects of data from our neonatally deafened and chronically stimulated animals suggest that mechanism(s) other than direct depolarization may mediate at least part of the conservation of SG neurons *in vivo*. First, the increases in neuronal density appear to be more broadly distributed throughout the cochlea than would be expected with discrete activation produced by a closely spaced pair of bipolar scala tympani electrodes as employed in these experiments. Yet, increased survival of neurons is *not* seen when electrodes are implanted but not activated, indicating that vascular changes and inflammatory processes which accompany implantation alone are not sufficient to promote survival (Leake et al., 1991). Moreover, the extent and spatial distribution of neurotrophic effects were not diminished when the intensity of stimulation was reduced from 6 dB in earlier experiments to only 2 dB above EABR threshold (Leake et al., 1992). These results suggest that there may be subthreshold effects of the electrical fields generated by the implanted electrodes or other factors that ameliorate the slow, retrograde degeneration of spiral ganglion neurons which otherwise is progressive for years after deafening (Leake and Hradek, 1988). Moreover, although we have demonstrated highly significant effects of electrical stimulation in promoting neural survival, SG survival is still far from normal in our chronically stimulated animals. We are thus very interested in exploring other potential neurotrophic factors, which may further augment neural survival when used in conjunction with electrical stimulation.

It seems likely that the key intracellular signaling mechanisms and pathways underlying the survival-promoting effects of electrical stimulation and other neurotrophic agents can be most efficiently investigated in cell culture preparations. Research on cultured SG neurons by Green and co-workers (Hegarty et al., 1997; Hansen et al., 2001) has demonstrated that neuronal survival is supported *both* by membrane depolarization and by neurotrophins: Their research has shown that the survival-promoting effect of depolarization is mediated by L-type voltage gated Ca^{2+} channels and involves multiple distinct signaling pathways, including 1) an autocrine *neurotrophin* mechanism; 2) cAMP production; and 3) CAM kinase-mediated phosphorylation of CREB. The neurotrophins BDNF and NT-3 are expressed by SG neurons and promote survival by an autocrine mechanism that is *additive* with the survival-promoting effect of depolarization.

Over the past several years, a number of *in vivo* studies have shown that several neurotrophic factors (usually administered via perilymphatic infusion) can reduce SG loss following deafness. The best-characterized neurotrophic factors are members of the nerve growth factor (NGF) family of proteins, and are called neurotrophins. Neurotrophins include NGF, brain-derived neurotrophic factor (BDNF), neurotrophin-3, and neurotrophin-4/5, each of which binds to specific high-affinity receptors, the Trk family of receptors. Neurotrophins are particularly relevant to our studies in neonatally deafened animals because they regulate neuronal differentiation and survival during development (Korsching, 1993, Gao et al., 1995) and also are known to protect neurons from injury and toxins in adults (Apfel, 1991; Hefti, 1986; Kanzake et al., 2003, Miller et al., 1997; Yan et al., 1992, Zheng et al., 1995).

In addition to our previous studies with the systemically administered neurotrophic agents GM1 ganglioside and desmethyldeprenyl, we are now initiating a new experimental series in which BDNF will be directly infused into the cochlea, in conjunction with chronic electrical stimulation in neonatally deafened cats. As mentioned

above, both electrical stimulation and BDNF are potent neurotrophic factors that promote survival of SG neurons, and recent work indicates that BDNF synthesis within SG neurons is constitutive and ***not*** activity-dependent (Yan et al., 1992; Hansen et al., 2001). Therefore, BDNF and electrical stimulation delivered concurrently should elicit survival-promoting effects that are relatively independent and ***additive***. For these studies, we will use scala tympani electrodes that are adapted to incorporate a drug delivery system. Such systems have been described and successfully applied in guinea pigs by a number of research groups (e.g, Shepherd, 2003). Most of these studies have utilized a Nucleus banded array that incorporates a cannula to permit delivery of neurotrophic agents directly into the basal scala tympani of the guinea pig (Shepherd and Xu, 2002; Paasche et al, 2003, Shepherd et al, 2005). The recent Shepherd et al. study (2005) demonstrated limited additive effects of electrical stimulation and concurrent BDNF administration, but the implanted electrodes were positioned in the extreme base of the cochlea. With these studies in mind, we have developed a new feline electrode capable of optimized chronic stimulation (4 active electrodes delivering electrical stimulation across a broad region of the cochlear spiral) and simultaneously delivering neurotrophic agents directly into the scala tympani. We hypothesize that this device will promote even greater neural survival, while at the same time accurately modelling the devices currently used in human implant recipients.

During the past quarter we completed component testing and prototype fabrication in preparation for implantation of a feline cochlear implant array combining electrical stimulation and therapeutic drug delivery. Eventually, we plan to deliver chronic electrical stimulation in combination with continuous intracochlear infusion of Brain Derived Neurotrophic Growth Factor (BDNF) for periods of up to six months. This report includes a description of the prototype devices built to date, documentation of long term *in vitro* testing to confirm the integrity of the drug delivery subassemblies, and a detailed description of the protocol for preparing the BDNF solution. It also details the procedure for filling and priming the osmotic pumps in a sterile environment. Finally, this quarterly report describes implantation and initial drug delivery using this prototype system in a juvenile cat.

DEVELOPMENT OF ELECTRODES WITH INTEGRATED DRUG DELIVERY

As presented in previous reports we have designed an improved intracochlear electrode for use in chronic studies. The primary objectives for the new design include an updated geometry that more accurately models current cochlear implants used in human subjects and the incorporation of a chronic drug delivery system driven by an implantable osmotic pump. The first prototypes of this new electrode have been completed and an example of an eight contact array is shown in Figure 1. The following report will focus on the development and testing of the drug delivery system intended for implantation in cats four to eight weeks in age and will describe implantation of the first animal in this experimental series.

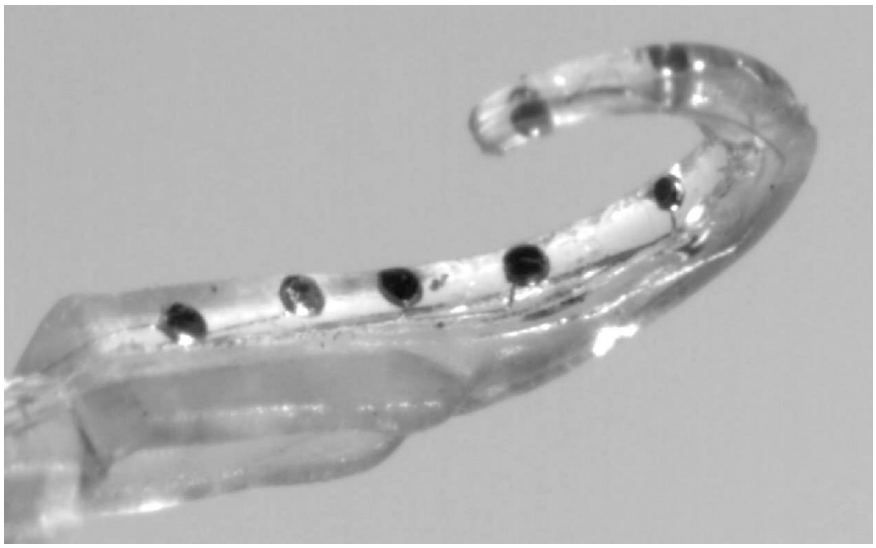

Figure 1. An eight channel feline electrode fabricated to model the designs of cochlear stimulating arrays used in human subjects is shown at the left. The electrode incorporates a drug delivery catheter which terminates in a protected channel extending from the round window 3.5 mm into the basal cochlea. An Alzet ™ miniature osmotic pump will act as a drug reservoir and provide constant flow for up to 30 days.

The drug delivery system consists of three parts. First, a reservoir that contains the therapeutic agent must be small enough to be tolerated by a kitten 500-600 grams in weight and must be capable of delivering the drug at a continuous rate over a period of up to six months. Implantable mini osmotic pumps (Alzet, Durect Corporation) were selected as the ideal candidates for this purpose. Their reliability has been demonstrate previously in many similar applications. The pumps can be surgically replaced at regular intervals, in order to provide a total of six months of delivery our experiments. In these initial trials brain derived neurotrophic factor (BDNF) will be used as the therapeutic agent.

The connection between the osmotic pump and the electrode is critical for reliable long-term function of the system. We chose medical grade vinyl tubing because it sturdily resists kinking, connects securely to the output port of the osmotic pump, and can be reliably attached to the silicone body of the intracochlear electrode using medical grade silicone adhesive (MED 1137, NuSil Technologies.) Finally, the distal drug delivery port must pass through the restricted volume of the round window into the scala tympani. For this segment of the system we used fine bore polyimide tubing.

As shown in Figures 2 and 3, a small hub was included in the electrode mold design to support the cannula leading from the intracochlear electrode carrier to the remotely located osmotic pump. The outside diameter of this male hub was sized to match the outer diameter of the 21 gauge output of the Alzet ™ osmotic pumps (0.030”/0.76 mm) so that a single piece of vinyl tubing (0.027”/0.68 mm ID intravenous medical tubing, www.scicominc.com) could be used to directly connect these two components. This hub also acts as a simple adaptor to allow coupling to the much smaller polyimide tubing (0.0064” OD, www.coleparmer.com) required in the scala tympani. The small bore polyimide tube (7.0 mm in length) was placed in the upper half of the electrode mold prior to injection molding. To prevent back-filling of the polyimide tubing during the injection molding process, a small plug of MED 1137 silicone was formed at each end of the tube and cured. Next, the tubing was set in the heated mold and tacked in place with silicone (MED 4011), and the mold was cooled to room

temperature. To maintain an open well for the terminus of the drug delivery catheter a small drop of partially hydrolyzed polyvinyl alcohol (PVA, Celvol 513, Celanese) was formed over the tip of the polyimide tube and in contact with the surface of the mold (see figure 3.) Thus, when the molded electrode carrier was removed from the mold and the PVA was dissolved by soaking in water, a small cavity or well remained around the tip of the catheter. This cavity is continuous with the larger cavity formed in the undersurface of the electrode by the ridge of metal running through the basal portion of the mold (see single arrow in Figure 2). In this way the terminus of the drug delivery tube is protected from physical blockage and the molded silicone channel leads to the apical turns of the scala tympani permitting direct infusion of the drug throughout the cochlea. The end of the polyimide tube was then clipped off to open the tube.

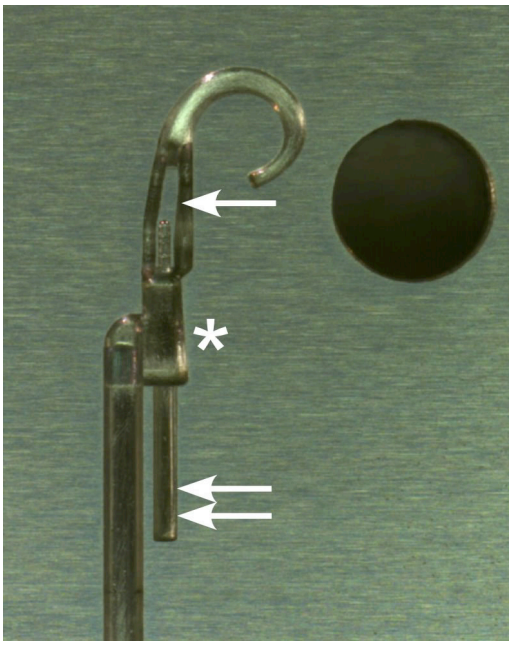

Figure 2. The primary mold features that form the drug delivery components of the electrode are shown. To minimize the possibility of kinking the catheter or tracking infection from the subcutaneous exit site for the electrical stimulation cable, the electrical leads and the catheter are separated at the round window (*). The drug delivery catheter extends in a straight path through the round window and the electrical leads are offset. A small section of the polyimide tubing will exit the intracochlear section of the electrode and connect with the osmotic pump via larger vinyl tubing. The interface with this larger tubing is made with a molded silicone sleeve (double arrow). The machined bar in the basal section of the mold (single arrow) forms a channel in the undersurface of the electrode that will provide a protected chamber for the tip of the polyimide catheter and facilitate reliable diffusion of the drug into the scala tympani.

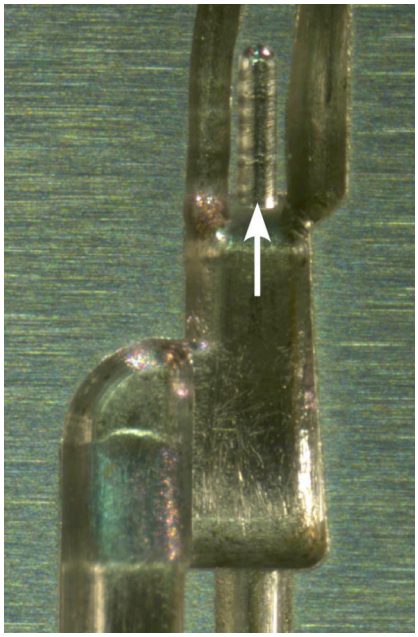

Figure 3. A fine slot was machined into the bar shown in Figure 2 to accurately position the distal end of the drug delivery catheter. In the fabrication sequence, the tip of the catheter is plugged with a small droplet of silicone adhesive. The catheter is then placed in the slot, tacked in position with silicone, and the remaining volume of the slot is filled with polyvinyl alcohol (PVA). Thus, when the mold is filled, the volume of the bar and catheter slot are protected from silicone. When the electrode is removed from the mold, the PVA is melted and washed away with hot water and the tip of the catheter is cut to expose the open lumen.

Figure 4 illustrates the polyimide tube molded into the silicone carrier. The opposite end of the polyimide catheter was exposed by amputating the last 2 mm of the hub containing the catheter (see double arrow in Figure 2). A piece of vinyl tubing (10 cm in length) was slipped onto the hub and attached with MED 1137 adhesive. The end of this vinyl tubing was left open to permit pre-filling with BDNF prior to attachment of the osmotic pump.

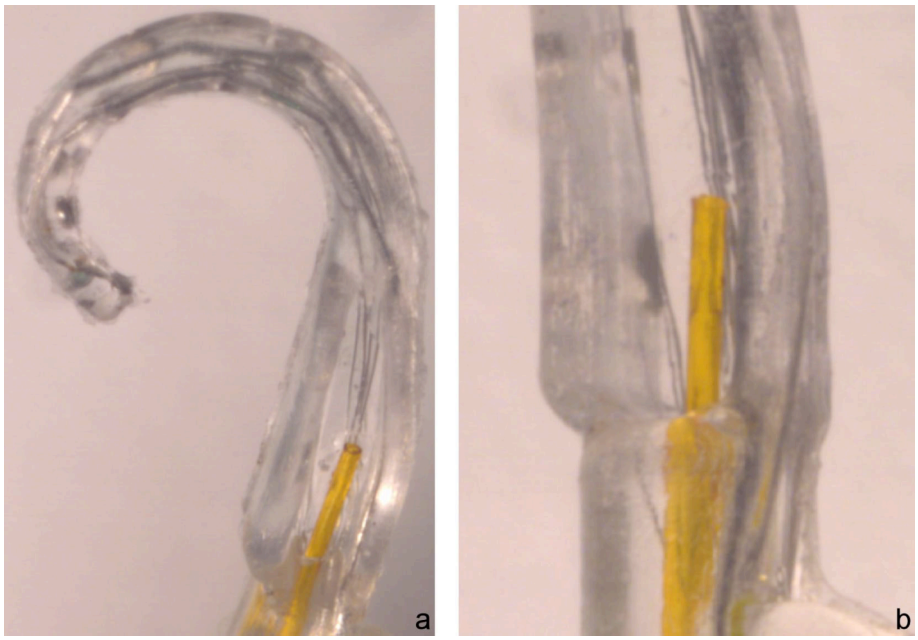

Figure 4. These micrographs illustrate the molded chamber that is formed in the underside of the electrode and the terminus of the fine polyimide catheter.

COMPONENT TESTING

During the last year we conducted a series of extensive tests to evaluate the long-term reliability of the drug delivery system components described above. The following is a summary of those evaluations. To examine the strength and reliability of the seal between the components of the drug delivery system we directly tested the connection between the polyimide and vinyl tubing using both pull and pressure tests in a series of 30 samples. In the first test we prepared 15 samples by joining pieces of vinyl tubing (30 mm in length) with a similar length of polyimide tubing. Each piece of tubing was thoroughly cleaned with ethanol prior to attachment. Fifteen millimeters of the smaller polyimide tubing was inserted into the vinyl tubing and the surrounding space was filled with MED 1137 elastomer. Care was taken to ensure that the elastomer did not occlude the tip of the polyimide tubing and the silicone was cured overnight prior to testing. To evaluate the possibility that prolonged implantation might weaken this connection we tested the tubing assemblies prior to soaking in sterile saline at 38° C and after soaking for 14 days, 1 month, 3 months, and 6 months. Pull testing consisted of firmly clamping each end of the tubing assembly and pulling the assembly apart until failure occurred. The results are presented in Table 1 below.

Time, Incubated in 0.9 % Sodium Chloride at 38° C

	0	14 days	1 month	3 months	6 months
Sample 1	140*	200*	200*	200*	180*
Sample 2	130*	200*	200**	200**	180**
Sample 3	120*	200*	200**	200*	200**

Failure modes
* Polyimide tubing stretch
** Two tubes pull apart at silastic junction

Table 1. Reliability of the joint between the fine polyimide tubing and the larger vinyl tubing using MED 1137 adhesive was tested by measuring the pull strength (in grams) over a period of 6 months. The force recorded at failure and modes of failure are shown in the table above.

In a second test we used compressed air to simulate the force generated by the osmotic pump in the presence of backpressure. This could occur if the lumen of the polyimide tubing became partially or completely blocked. Fifteen test samples were fabricated as described above and the tip of the polyimide tubing was sealed with MED 1137. The vinyl end of the tubing assembly was slipped over a 21-gauge blunt tip stainless steel hypodermic needle to simulate connection to the osmotic pump. In this way both the connection between the two pieces of tubing and the connection to the osmotic pump were tested. Each assembly was tested under water with increasing pressure to reveal leakage and the location of failure was recorded for each trial. Again, three samples were tested prior to soaking at 38° C and three samples were tested at 14 days, 1 month, 3 months and 6 months intervals. The results of this test are presented in Table 2.

Time, Incubated in 0.9 % Sodium Chloride at 38° C

	0	14 days	1 month	3 months	6 months
Sample 1	> 40 psi	> 40	> 40	> 40	> 40
Sample 2	> 40 psi	40	> 40	> 40	> 40
Sample 3	> 40 psi	35	> 40	> 40	> 40

Table 2. To evaluate the possibility of leakage at the tubing junctions, samples of polyimide and vinyl tubing were joined and connected to 21 gauge stainless steel tubing equivalent in dimension to the output tube of the Alzet ™ osmotic pump. This assembly was pressure tested over a period of 6 months as shown above. Values represent pressure (psi) at failure.

To verify pumping rates, and further test the connection between the diffusion moderator and vinyl tubing, we tested the Alzet ™ Model 2001 (specified 1 µL/hr, 7 days), Model 1002 (spec. 0.25 µL/hr, 14 days) and Model 2004 (spec. 0.25 µL/hr, 28 days) pumps in sterile saline. A dry weight for each pump and diffusion moderator (DM) was recorded and the pumps were filled with a solution of blue dye in sterile saline using a syringe as directed in the manufacturer's instructions. The DM was reinserted into the filled pump and a final weight was tabulated. The net weight (mg) of the filled

pump corresponds to the fill volume (µL) of the pump. Sixty cm of vinyl tubing was connected to each osmotic pump and the entire system, except the tip of the vinyl tubing, was placed in a sealed glass reservoir of sterile saline and maintained at a temperature of 38° C. The pumping rate was calculated by recording the length of the blue dye column in the tubing each day. Based on the internal diameter of the vinyl tubing (0.69 mm), the volume of one cm of tubing equals 3.69 microliters. The output rate for each pump was plotted over time to document the time needed to prime each model, the daily pumping rate for each pump, and the total output amount and duration under the test conditions.

The output rate for each model osmotic pump considered for use in the chronic implant study was measured *in vitro*. Figures 5 and 6 illustrate the results of these tests (n = 12). With the exception of one manufacturing lot, the osmotic pumps performed as specified and their output was constant over the expected duration of each model. All pumps required 24-48 hours immersion in saline to reach their specified output level. In most cases the output after 24 hours was 70-80% of the final output. These data confirm that the recommended 24 hour priming period in saline is sufficient to ensure reasonably effective pumping immediately upon implantation.

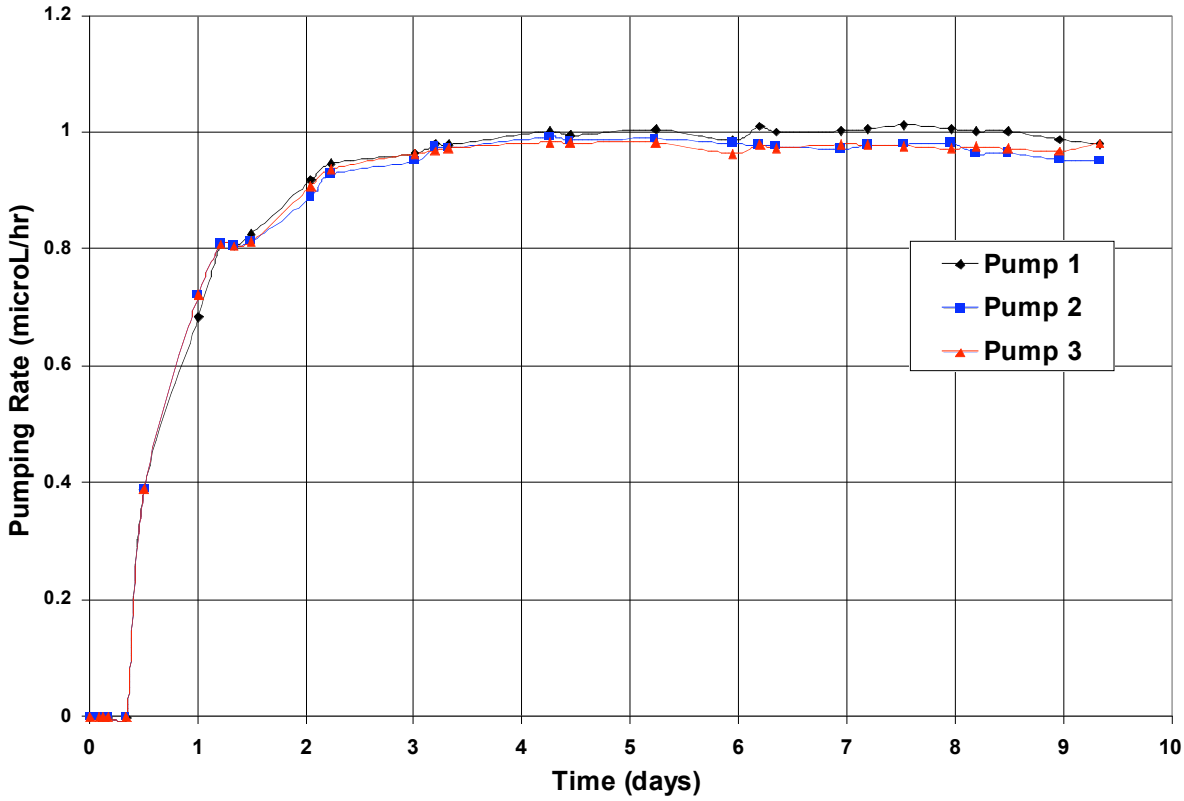

Figure 5. The flow rate of the Alzet ™ osmotic pumps was measured to ensure accurate flow and evaluate the priming period needed prior to implantation. The output graph in this figure is a plot of flow rate versus time for the smallest Alzet ™ pump, model 2001, designed for seven day use following a 24 hour priming period.

The output rate of two of the tested pumps was unusually high (100-200% above normal) during the first 24 hours in the saline bath. However, this initially high pumping rate was limited to the priming period and did not appear to affect the long-term performance of either pump. In contrast to the accurate flow rate of three of the four manufacturing lots tested, the pumping rate of one lot of 28-day pumps (Model 2004, Lot #10095-04A) was consistently higher than specified (0.25µl/hr). As shown in Figure 6 the mean output of this set of pumps was 0.38µl/hr, 52% higher than the specified rate and the tested value for the lot (0.25µl/hr) in the manufacturer's quality assurance certification. In addition, the output of this group of pumps decreased beginning at 24 days. A second set of pumps was provided by the manufacturer (Model 2004, Lot #10034-01), tested, and found to be within specification.

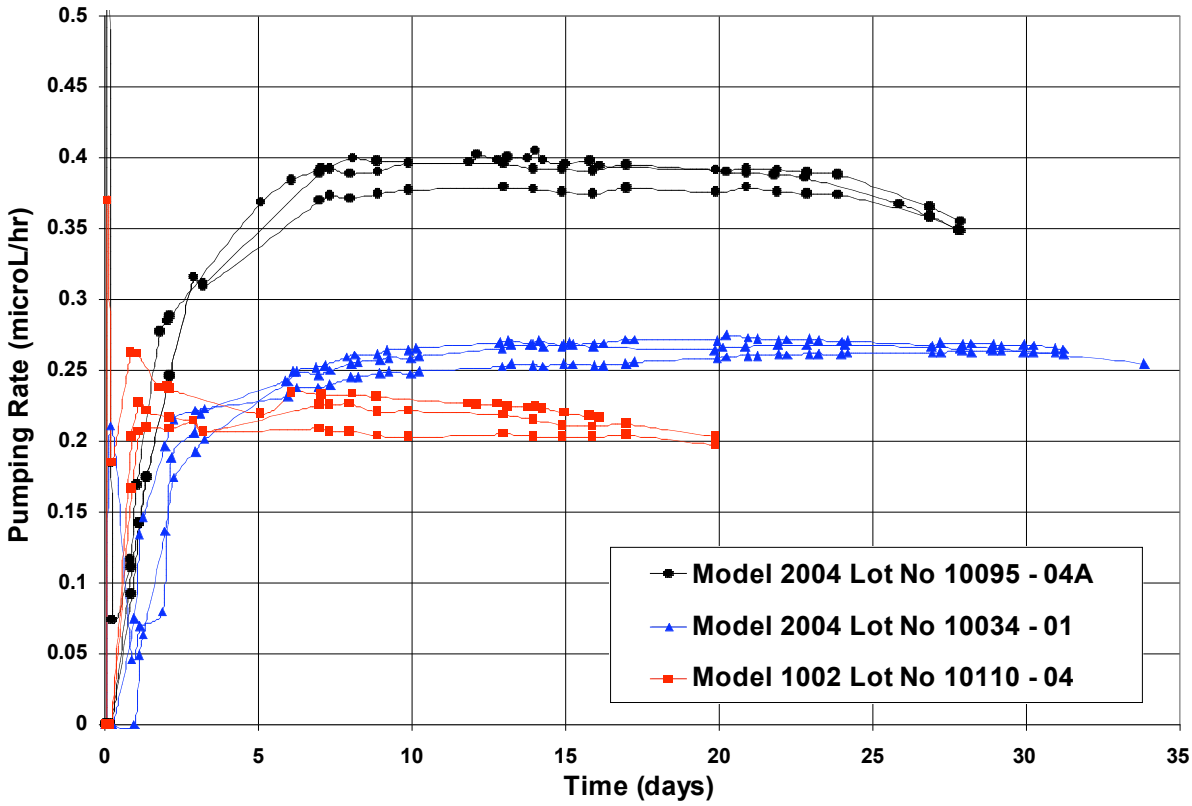

Figure 6. Flow rates for one lot of 14 day pumps and two lots of larger 28 day pumps are shown above. The flow rate of the first lot of 28 day pumps was significantly above the specified rate and the output of all three pumps tested in this lot decreased and stopped before the required 28 day period. In contrast, 28 day pumps from a second lot were within specification for both the flow rate and duration.

PREPARATION FOR IMPLANTATION

Prior to implanting the drug delivery system, a sterile solution of BDNF in artificial perilymph was prepared, the osmotic pump was loaded with the BDNF, and the pump was primed for a minimum of 24 hours under aseptic conditions to ensure that effective infusion begins immediately upon implantation (see Figures 5 and 6.) It is critical that the osmotic pump and all tubing be fully primed at the time the system is implanted to

avoid tissue or protein accumulation at the tip of the catheter which can lead to blockage of the catheter lumen and subsequent failure of the system.

Artificial perilymph was prepared as follows: 176 mg of sodium Bicarbonate ($NaHCO_3$, 84.01 g/mol) was added to 100 mL of Ringers solution (147 mM Na^+, 4 mM K^+, 5 mM Ca^{++}, 156 mM Cl^-.) Blood was then drawn from the kitten to be implanted, centrifuged and separated. The supernatant (serum, protein layer) was extracted and diluted 1:1 with the ringers/bicarbonate solution. This formulation was then passed through 0.45 µm and 0.2 µm sterile filters. A total dilution of 1:30 (serum:ringers/ bicarbonate) was then prepared, adjusting the blood protein concentration to approximately 200 mg/dL. This Ringers, bicarbonate, serum formulation constitutes the artificial perilymph (AP) solution used in this study. The AP was then passed through a NALGENE sterile filter and separated into several sterile microtubes. Approximately 1.5 to 2 ml was placed into each vial. The microtubes and contents were then quickly frozen in a bath of liquid nitrogen and stored to be used for future pump filling and replacement.

To fill the osmotic pumps, 1.5 ml of the AP and 100 µl of BDNF solution (1 mg/ml) were thawed and combined in a Class II A/B3 Biological Safety Cabinet under UV light using sterile techniques. This combination of AP and BDNF generates a concentration of 62.5 µg/ml (equivalent to that used by Shepherd et al. 2005.) The pumps where then attached to vinyl tubing and placed in a sterile incubation jar for the 24 hour primping period. The pumps were incubated in sterile saline and both the pumps and remaining AP:BDNF formulation were transported to an oven and kept at 38° C until implantation (24-40 hours). This priming process ensures immediate delivery upon implantation. The vinyl and polyimide lumens of the implanted device are pre-filled with the same formulation at the time of surgery. To test the sterility and techniques of our filling procedure, a pump was filled and incubated for 14 days. The formulation remaining in the pump reservoir was extracted and added to a sterile incubation medium. This tested for the growth or presence of any contaminants in the formulation. Both positive and negative controls were run concurrently with the pump samples. No indication of contamination was seen in any of the samples tested.

IMPLANTATION AND PUMP REPLACEMENT

Recently, this new electrode/drug delivery system was implanted in a 4-week old neonatally deafened kitten. The cochlear implant procedure was nearly identical to that used for all previous devices. However, prior to implantation of the device, the tubing that was to be attached to the osmotic pump was pre-filled with AP:BDNF solution and the distal end of the tubing plugged with bone wax. The kitten was anesthetized with inhaled isoflurane, body temperature was maintained by a water circulation blanket thermostatically controlled by a rectal probe and heart rate, respiration, oxygen saturation, exhaled carbon dioxide were continuously monitored. Using strict aseptic surgical procedures, a postauricular curvilinear incision was made behind the left ear, the strap muscles were reflected to expose the auditory bulla, and the bulla was opened to access to the round window. The intracochlear electrode was then inserted into the scala tympani and secured in place with a dacron cuff attached to the promontory with tissue adhesive (Histoacryl™). The electrode and the tubing for the osmotic pump were routed under the temporalis muscle, and additional dacron cuffs were used to secure

the them to the parietal skull (one cuff just superior to the bulla and another near the midline). Near the midline a silicon rubber pouch with a redundant loop of the cable forms a 90^{o} angle to route the electrode caudally. The cable was passed subcutaneously under the skin at the nape of the neck and the connector was externalized between the scapulae. A dacron sleeve proximal to the connector was sutured subdermally to secure the connector near the exit site.

After the cochlear implant and its cable were in place, the pre-filled tubing for the osmotic pump was cut to an appropriate length and the pump attached. A loop was created in the tubing and sutured in place just lateral to the midline point where the stimulation cable turns caudally. The pump was then tunneled subcutaneously and positioned postauricular to the ear contralateral to the implanted ear. The incision was sutured closed in anatomic layers using 3-0 Dexon and 4-0 Vicryl. The surgical procedure required about three hours. The animal was then declawed and EABR thresholds determined. Buprenorphine and carprofen (a nonsteroidal anti-inflammatory analgesic agent) were administered for pain. At the initial implantation when the animal was 4 weeks of age, we used the smaller model of osmotic pump with a 2 week capacity. After the 2-week period had elapsed and the pump appeared to be very well-tolerated, a second short (20 minute) surgical procedure was conducted to remove the depleted pump and replace it with the larger model osmotic pump, with 30 day drug delivery capacity. The second pump has also been extremely well tolerated with no swelling or irritation in the area. Chronic electrical stimulation was initiated a few days after the second procedure and longitudinal EABR data are being collected. We plan to sacrifice this animal at 8 weeks of age, an age at which we have control data for many neonatally deafened animals without BDNF treatment. In this new experiment, it seems appropriate to first complete a shorter-term pilot series and conduct histological studies evaluating the effect of BDNF delivered over the period from 4-8 weeks of age (e.g., to ensure that BDNF is effective in cats, that the BDNF delivery system is functional, etc.)

If initial results are promising, we plan in future experiments to deafen animals, and to wait either a minimum of 48 hours (or up to four weeks) after the neomycin treatment is terminated and then implant the BDNF electrode. Waiting 48 hours ensures that implanting the ear does not ameliorate the effects of the neomycin (e.g. perilymph leakage, inflammation). Waiting longer in other subjects will test the effect of delayed treatment. In the next planned series, the miniosmotic pump will be replaced after an initial period of 2 weeks and at one-month intervals thereafter to continue treatment concomittant with electrical stimulation from the cochlear implant over periods of 3-6 months. Half of the animals in the initial chronic stimulation series will be studied immediately after 3 months of BDNF treatment; half of the group will be maintained for a subsequent period of 3 months with electrical stimulation alone to determine whether any observed neuroprotective effects of BDNF are maintained over a longer period after the BDNF treatment is terminated. This protocol is designed to address the important issue raised by Gillespie et al., (2003) who reported that although BDNF promotes neural survival *in vivo* over the short-term, cessation of BENF treatment actually may lead to accelerated neural degeneration. If initial chronic studies are successful and multiple changes of the pumps are tolerated well, we would then prolong periods of BDNF treatments concomitant with electrical stimulation to assess longer-term effects.

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WORK PLANNED FOR NEXT QUARTER

1) Two neonatally deafened subjects will complete chronic electrical stimulation and will be studied in terminal acute electrophysiological studies recording from the inferior colliculus.

2) Data analyses of spiral ganglion survival, and cochlear nuclear morphology along with electrophysiological data will continue in the new experimental group of subjects that have been deafened at 30 days rather than neonatally (including the new subject studied acutely last month). The goal of this series to examine possible critical periods in the anatomical effects of deafness and chronic electrical stimulation on the cochlea and cochlear nucleus.

3) During this next quarter, another normal adult cat will be studied in an acute electrophysiological experiment to obtain control data utilizing the 32-channel NeuroNexus probes and our new 8 channel feline electrode with modiolar-facing stimulating contacts. A brief acoustic calibration procedure will be conducted during which a penetration site in the IC is selected and an ideal penetration depth determine based on the range of characteristic frequencies recorded, which must encompass the range of frequencies accessed by the intracochlear implant electrode. Next, the animal will be deafened by an injection of kanamycin followed by IV infusion of ethacrynic acid to effect, as indicated by elevation of ABR thresholds. Finally, an 8-channel UCSF cat electrode will be implanted and responses to electrical stimuli will be recorded. Data collection will focus on electrical spatial tuning curves, masking, SAM modulation depth and 2 channell interaction protocols.

4) During the current quarter, one subject in the 30-day deafened series damaged his device failed to meet the criterion 6 months of stimulation. Although the data from this subject will still be usable, we will implant one more subject durng the next quarter that will hopefully complete the full protocol.

5) One of our female cats is pregnant and a litter of kittens is expected during the next quarter. Two more kittens will be studied in pilot experiments to examine the effects of BDNF on spiral ganglion survival by infusing BDNF directly into the cochlea in neonatally deafened animals. This pilot study is a first step toward eventually evaluating the effects of combined BDNF treatment and electrical stimulation of the cochlea.

6) Studies of the human cochlea will continue, with current analyses directed toward refining mathematical functions that will define represented frequency along both the organ of Corti and spiral ganglion as a function of angle of rotation from the round window.

2006 ARO ABSTRACTS:

Behavioral-Neural Model for Temporal Integration in the Deaf Cat
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Temporal integration (TI) allows lower (more sensitive) detection thresholds when the duration of a stimulus is increased in normal hearing subjects. However in hearing-impaired subjects, an increase in stimulus duration has a smaller effect on threshold, i.e., there is less TI. This poster describes a model of TI based on behavioral and neurophysiological experiments in the deaf cat.

Cats (n=3) deafened neonatally by intramuscular injections of ototoxic antibiotics were trained to avoid a mild electrocutaneous shock when single or trains of electrical biphasic rectangular pulses (5.0 ms/phase) were applied to the cochlea via a UCSF feline auditory prosthesis. Psychophysical detection thresholds (50% avoidance) and reaction times were estimated as functions of stimulus duration. Electrophysiological responses of single neurons in the central nucleus of the inferior colliculus (ICC) were evoked using sinusoidal pulse trains (100 Hz), which varied in intensity and duration. Only data for neurons (n=42/76) that responded with sustained discharges to 300 ms pulse trains at +2 dB SL are presented in this poster.

The results include: 1) Psychophysical thresholds decreased by about 7 dB for the longest duration stimulus (1.0 s) compared to the shortest duration stimulus (0.01 s) for each cat (t-test; $p<.001$); 2) the slope of the mean behavioral TI function was -1.2 dB/doubling of stimulus duration; 3) for each neuron, the number of spikes increased monotonically with stimulus duration (ANOVA; $p<0.001$); 4) neuronal response magnitude was dependent on stimulus intensity (ANOVA; $p<0.001$); and 5) the slopes of mean neuronal TI functions were equivalent to the slopes of behavioral TI functions in the deaf cats but were reduced compared to TI reported for normal hearing cats.

Supported by NIH/NIDCD Contract N01-DC-3-1006.

Estimating Represented Frequencies for Cochlear Implant Electrodes in Human Temporal Bone and Imaging Studies
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Greenwood's frequency-position function (Greenwood, 1990, JASA 87) has been used to estimate frequencies of cochlear implant (CI) stimulation sites both in temporal bone studies and in imaging studies of living CI recipients. The function calculates frequency as percent of organ of Corti (OC) length, but there is no accurate method for estimating OC length in such studies. Also, many CIs place electrodes near the modiolus to target the spiral ganglion (SG), and the SG frequency map is different from that of the OC (Sridhar et al, ARO 2005). Our goal is to develop better methods for estimating frequencies of CI electrodes.

Cadaver cochleae (n=9) were fixed <24 hrs postmortem, stained with osmium tetroxide, microdissected, decalcified briefly, embedded in epoxy resin and examined in surface preparations. In digital images, the OC and SG were measured and radial nerve fibers were traced to define frequency-matched points along the two structures. Expressed as percent of length, the data sets were highly correlated and best fit by a cubic function that allows derivation of SG frequency from Greenwood's equation.

The mean OC length was 33.13 mm, but the mean SG length (center of Rosenthal's canal) was only 13.69 mm, and that of the modiolar wall adjacent to the SG (optimum position of CI electrode) was 15.49 mm. Both OC and SG lengths were correlated (r^2=0.78, 0.88 respectively; $p<0.005$) with cochlear size (average of the maximum basal coil diameter and the orthogonal diameter). This finding may allow estimation of OC and SG length in imaging studies. Data also suggest that frequency vs. angular position is relatively constant, but insertion distance is correlated with cochlear size (e.g., 450° from the round window the mean OC frequency is 604 Hz with a range of only 0.4 octave, but insertion distance at 450° varied from 20.9 to 24.8 mm). Accurate frequency maps for OC and SG should allow better matching of CI processor filter bands to stimulation sites.

Work supported by NIDCD Contract N01-DC-3-1006

Effects of Auditory Deprivation and Electrical Stimulation in the Developing Auditory System
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This study was designed to examine the effects of different deafening protocols on the anatomical organization of the cochlea and cochlear nucleus (CN). It explores the possible role of developmental "critical periods" in the effects elicited by electrical stimulation from a cochlear implant. Cats were deafened by daily neomycin injections for 18-26 days starting at 30 days of age. These animals were studied at 8-9 weeks of age or after several months of electrical stimulation (325 pps/60Hz AM) beginning at 8-9 weeks. Data from the stimulated and unstimulated groups were compared to neonatally deafened animals matched for age and duration of stimulation/deafness.

In both neomycin deafened groups studied at 8-9 weeks, spiral ganglion cell (SGC) survival was already significantly reduced, despite the fact that animals in the 30-day deafened group had a brief period of normal hearing before neomycin treatment was initiated and were studied only 1-2 weeks after deafness. Electrical stimulation delivered over 18-30 weeks significantly enhanced SGC survival in the group deadened at 30-days, with maintenance of 15-20% higher SG cell density in the stimulated ears. The neonatally deafened group showed a similar increase in SGC survival with no significant difference between the 30-day deafened and neonatally deafened groups.
Measurements of CN size, however, revealed significantly higher values in the group deafened at 30-days when compared to neonatally deafened cats at 8-9 weeks of age. There was also a trend toward larger CN size in the 30-day deafened group studied after electrical stimulation, as compared to the neonatally deafened, stimulated group. These findings suggest that a period of normal auditory experience may be significant in lessening degenerative changes in the central auditory system after early-acquired deafness (as compared to the effect of deafening immediately after birth).

Work supported by NIDCD Contract N01-DC-3-1006 and R01 DC000160.

Selectivity of Electrical Activation of the Auditory Midbrain by a Cochlear Implant is Degraded Following Long-Term Deafness in Cats
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Selectivity of neural activation is an important factor in functional independence of channels in a multichannel cochlear implant (CI). This study examines the effects of neonatal deafness on spatial selectivity of electrical stimulation delivered by intracochlear biplolar electrodes in the central auditory system. Electrically evoked auditory brainstem response (EABR) thresholds and neural response thresholds in the inferior colliculus (IC) were estimated in neonatally deafened cats studied after varying durations of deafness. Acutely deafened adult cats served as controls. Threshold distributions across the tonotopic gradient of the IC were analyzed to determine the spread of excitation (spatial selectivity/tuning) and the dynamic range (suprathreshold intensity at which activation is elicited simultaneously at all recording sites across the two subnuclei of the IC) of responses to intracochlear electrical stimulation. In addition, spiral ganglion cell (SGC) densities were determined in each cat.

Long-deafened animals (duration of deafness >2.5 yr) with severe cochlear pathology (mean SGC density <6% of normal) had significantly higher EABR and minimum IC thresholds than animals deafened <1.5 yr (mean SGC density ~45% of normal). Also, the spatial extent of electrical excitation was significantly broader and the dynamic range significantly reduced in long-deafened animals compared to the other groups. However, regardless of the duration of deafness, the cochleotopic organization was maintained in both the external and the central nucleus of the IC.

These data suggest that long-term auditory deprivation results in a significant degradation of spatial selectivity of intracochlear electrical stimuli in the central auditory system. These changes likely contribute to poorer speech discrimination performance in prelingually deafened human cochlear implant users that are implanted as adults after long durations of deafness.
Supported by NIH NIDCD Contract N01 DC-3-1006.