KOPF CARRIER

September 2017

Kopf Carrier #91

PUBLISHED BY DAVID KOPF INSTRUMENTS ■ TUJUNGA, CALIFORNIA

# Optrode Implantation into the Rodent Brainstem to Evaluate Information Coding of Gustatory Stimuli

Joshua D Sammons, PhD, and Olga Escanilla, MS
4400 Vestal Parkway East
Binghamton NY 13902-6000

Dr. Joshua Sammons (jsammons@binghamton.edu) is a post-doctoral research associate and Ms. Olga Escanilla (oescani1@binghamton.edu) is a PhD candidate in Dr. Patricia Di Lorenzo's lab. They investigate methods of information transfer in the gustatory brainstem of Sprague Dawley rats.

## Abstract

The gustatory brainstem is one of the more difficult areas from which to record neuronal activity in awake, behaving rodents for several reasons. One major reason is that the brainstem is a hub for autonomic functions and implantation of large bundles of electrodes can have fatal consequences. Additionally, neurons in the gustatory nuclei are much smaller than in other gustatory regions such as the cortex. Here, we provide a methodological overview of two successive surgical techniques that allow for the use of optogenetics during electrophysiological experiments in the gustatory brainstem of Sprague Dawley rats. Optogenetics is a technique that uses light-sensitive proteins called opsins to stimulate or inhibit neuron activity. The first surgery in the protocol introduces a viral vehicle to deliver a gene for the opsin of choice into the brain region of interest. The second technique details implantation of an optrode into the nucleus of the solitary tract. These optrodes consist of 8–16 microwires to record neuron activity and a fiber optic implant to manipulate the neural activity through light delivery.

Key words: Kopf, brainstem, electrophysiology, optogenetics

## 1. Introduction

The study of electrical excitability of cells within an organism's nervous system started in the late 18th century when Luigi Galvani experimented with stimulating nerve fibers that caused muscle twitches in a dead frog leg (1). Today, electrophysiological methods are used in many fields of science and medicine (2–5). In biomedical sciences, one method with excellent temporal and spatial resolution is the extracellular recording of multiple individual neurons. This method utilizes microelectrodes implanted into the brain to monitor changes in extracellular electrical potential around each site. We use this method within the gustatory brainstem of awake, behaving Sprague Dawley rats as our model to understand how information is relayed within the brain. We further manipulate electrical potentials through optogenetics while the rat is performing desired tasks.

Optogenetics is a method of using light-sensitive proteins (opsins) to manipulate neural activity on a cellular level. It is a more versatile technique than electrical stimulation because specific neuron populations can be stimulated or inhibited within a region. Alternatively, specific axons originating from one region and terminating in a different region can also be manipulated (6,7). Using optogenetics, excitation of neurons can be achieved by expression and activation of channelrhodopsin, a light-sensitive sodium channel. Inhibition of neurons is achieved by expression and activation of halorhodopsin, a light-sensitive chloride channel, or archaerhodopsin, a light-sensitive proton channel. There are many variants of each opsin with different wavelength sensitivities. ChR2 is a common channelrhodopsin with maximum activation at 470 nm light. eNpHR3.0 is a common halorhodopsin with optimum light sensitivity at 589 nm light. Different wavelengths of light can be used with each opsin, though opsin activation would be decreased. For example, when testing the functionality of eNpHR3.0 for the first time, 532 nm light can be used. Though eNpHR3.0 is much less sensitive to 532 nm light than 589 nm light, the price of lasers with wavelengths around 589 nm is a large deterrent when setting up optogenetics in the lab. After the technique has been proven to provide useful data, the more expensive laser can be purchased.

The gene for an opsin can be inserted into the rat genome via a viral vehicle and expression of the opsin is usually observed within 2–4 weeks. After viral injection, a fiberoptic implant can be inserted into the region of interest and connected to a light source for light delivery and opsin activation. When recording neuronal activity in an area where optogenetic manipulation is desired, an optrode can be used. Two reasons for optrode use would be manipulation of one neuron subtype in the region of interest or synaptic manipulation in the region of interest. An optrode consists of a fiberoptic implant attached to an electrode consisting of one or more recording sites such as microwires. With an optrode, neurons can be optogenetically excited or inhibited with the fiberoptic implant while the recording sites record neuron responses. Here, we describe two surgical protocols used to prepare Sprague Dawley rats for electrophysiological experiments with optogenetic manipulation of neuron populations: injection of virus for opsin expression and implantation of optrodes into the nucleus of the solitary tract.

## 2. Materials

### 2.1. Biological Materials

2.1.1. Sprague Dawley Rats

7–8-week-old males, 9–10-week-old females

2.1.2. Viral reagents on ice

## 2.2. Apparatus

2.2.1. Stereotaxic Alignment System

Model 1900 stereotaxic alignment instrument (Patent number 6,258,103, Patent number 6,716,220), Model 1905 alignment indicator, Model 1915 centering scope, Model 1970 electrode holder, Model 1925 15° angle adapter, Model 1929-B rat gas anesthesia head holder

2.2.2. Syringes and Needles

26-G Luer lock needles, 26-G hubless needle (30-mm length, 30° bevel) (Hamilton #22026-01), 10-µL glass syringe with 26-G needle (Hamilton Model 1701 series), 1- and 3-mL disposable syringes

2.2.3. Virus Injection Surgical Tools

PE 20 polyethylene tubing (Intramedic #427406), Olsen-Hegar needle holder/scissors (Roboz #RS-7884), biodegradable sutures (Ethicon #VCP316H), syringe pump (Kent Scientific Genie TouchTM), biohazard bags (Bel-ArtTM F131660000), hair trimmer, rectal temperature probe and heating pad, 2 curved mosquito forceps (Roboz #RS-7101), 2 curved Graefe tissue forceps (Roboz #RS-7153), #55 Dumont tweezers (Roboz #RS-5063), #3 scalpel (Roboz #RS-9843), #15 scalpel blades (Bard-Parker #371153), 0.75-mm drill bits (Stoelting #514553), cotton swabs, gauze, sterile gloves, disinfectant wipes (sporicidin wipes)

2.2.4. Optrode Implant Surgical Tools

Hair trimmer, rectal temperature probe and heating pad, 2 curved mosquito forceps (Roboz #RS-7101), 2 curved Graefe tissue forceps (Roboz #RS-7153), 1 full curve Reynolds forceps (Roboz #RS-7211), #55 Dumont tweezers (Roboz #RS-5063), #3 scalpel (Roboz #RS-9843), micro-dissecting knife (Roboz #RS-6210), #15 scalpel blades (Bard-Parker #371153), 0.75-mm drill bits (Stoelting #514553), cotton swabs, gauze, stainless steel bone screws (FHC #40-77-8), sterile gloves, disinfectant wipes (sporicidin wipes)

2.2.5. Monitoring Tools

Physiological monitor (Kent Scientific PhysioSuite Monitoring Device), oscilloscope (Tektronix DPO3034), low-level AC amplifier (Grass Instruments #P511), speaker (Grass Instruments AM7B Audio Monitor)

### 2.3. Buffers and Reagents

2.3.1. Surgery

Mineral oil, 70% ethanol, xylazine, ketamine (controlled substance), buprenorphine (controlled substance), atropine, gentamycin, eye ointment (Altalube ointment), isosaline (0.9% NaCl in dH2O), lactated ringers, artificial saliva (14.8 mM NaCl, 22.1 mM KCl, 3.1 mM CaCl2, 0.6 mM MgCl2), taste stimulus (100 mM NaCl), Neosporin

## 3. Methods

## 3.1. Viral Surgery

Though more transgenic rat models are being generated annually, they are not yet that common. One of the most efficient methods of transgene expression in the rat model is through the use of viral vehicles. Rats can be obtained from many vendors; we obtain Sprague Dawley rats from Taconic Biosciences. Viral particles can also be obtained from multiple vendors with many optogenetic-related viruses being offered by the Deisseroth lab (Stanford University) and associated labs. All tools and the surgical area must be sterilized before surgery. Note: two methods of anesthesia are used in this manuscript:

ketamine/xylazine injection for viral surgery and isoflurane gas for optrode implantation. Either method can be used for each procedure, but tradition has shaped how our lab uses the two anesthesia methods.

3.1.1. Prepare Virus

Prepare 0.5 µL of virus (1×1012–1×1014 vcg/mL) per injection site per rat. Attach the 26-G hubless needle to one end of 0.5-m of PE 20 tubing and fill the needle and tubing with mineral oil. Place the other end of tubing onto the 10-µL syringe and pull the necessary volume of virus into the syringe. Note: the virus will not enter the syringe. No air pockets should exist to avoid inaccuracies in volumes dispensed. Attach the needle to the Model 1970 electrode holder.

3.1.2. Anesthetize Rat

Perform anesthesia using 0.1 mg/kg ketamine/0.014 mg/kg xylazine mixture with 0.1 mg buprenorphine to enhance the effects of the anesthetic and 0.1 mg/kg atropine to prevent pulmonary edema. About 30 min before surgery, give the rat a subcutaneous (s.c.) injection of buprenorphine solution using a sharp 26-G needle. Just before surgery, inject the ketamine/xylazine mixture into the intraperitoneal cavity using a fresh 26-G needle. Give an s.c. injection of atropine at the same time. Allow the rat to reach a surgical plane of anesthesia. Monitor vitals with a PhysioSuite Monitoring Device and provide a booster of one-third the initial anesthetic dose if the rat's vitals rise above the surgical plane of anesthesia. Though each rat is different, when under ketamine, the rat should have a heart rate under 325 bpm and respiration rate below 30 bpm when it is at a surgical plane of anesthesia.

3.1.3. Prepare Rat for Surgery

Shave the rat's scalp from ~1 inch behind the ears to just behind the eyes with the hair trimmer. Apply eye ointment onto the rat's eyes to prevent drying out. Insert a rectal temperature probe into the rat's rectum and maintain temperature at 37°C throughout the surgery by placing the rat on the heating pad. Place the rat into the stereotaxic device by placing the ear bars directly behind the zygomatic arch such that the head can swivel up and down, but not side to side. Ensure that the head is centered within the Model 1900 stereotaxic instrument. The zero on the stereotaxic device will generally line up between the 7- and 8-mm tick marks on the ear bars. Place the rat's teeth into the standard bite bar and tighten the bar until the snout is firmly fixed. Finally, sterilize the scalp with betadine three times, using a different stroke technique each time, and wipe clean with 70% ethanol.

3.1.4. Expose Skull

Using a sharp scalpel, make an incision on the scalp along the medial axis from the back of the skull to just anterior to bregma. Note: too much pressure will damage the skull. Brush the periosteum away from bregma, lambda, and the site(s) of interest with a cotton swab soaked with saline. Hold the skin away from the surgical site using a pair of curved mosquito forceps on either side of the site.

3.1.5. Level Head

Using the Model 1905 alignment indicator, ensure that bregma and lambda are on the same dorsal-ventral plane. If they are not, adjust the angle of the head until they are.

3.1.6. Drill Pilot Holes

Cite bregma using the Model 1915 centering scope and locate the site on the skull that is directly dorsal to the structure of interest. Mark the site with a fine-tipped marker or other indicator. If multiple structures are desired, locate and mark each structure. Drill a hole approximately 1.5–2 mm in diameter at each marked site. Ensure that the skull is cleared away but the surface of the brain is not damaged.

3.1.7. Inject Virus

Use the injection needle to cite bregma and then move above the pilot hole, ensuring that the coordinates are the same. If not, enlarge the pilot hole so that the needle can touch the brain surface. Use the brain surface as a reference and lower the needle to the appropriate depth so that the needle tip is within the target structure. This is done at a rate of 1 mm/min to ensure minimal damage to the brain. Wait an additional 1–2 min for the brain to settle and then inject the viral solution at a rate of 0.5 µL/min. After injection, wait for an additional 5 min for the viral solution to permeate through the tissue. If the needle is pulled up too soon, the virus will come as well and off-target expression will be observed. After injection, pull the needle out of the brain at a rate of 2–3 mm/min. Repeat this step for additional injection sites.

3.1.8. Close Wound and Perform Postoperative Care

Remove mosquito forceps. Depending on the incision length, use 3–5 stitches of a biodegradable suture to close the surgical site. Replenish fluid lost during surgery by injecting up to 3 mL lactated ringers. When the rat awakens, provide postoperative care to ensure efficient recovery.

**3.2. Optrode Implant**

Electrode implantation into the brainstem is a delicate process because it is a hub for autonomic functions and disruption of autonomic function can lead to death. To reach the gustatory nuclei of the brainstem with minimal damage, slow progression with constant monitoring of the electrical signal is optimal. Here, we describe a method for optrode implantation into the nucleus of the solitary tract with ~25° head tilt to avoid the sinus. We use homemade optrodes, but optrodes can also be bought.

3.2.1. Surgical Preparation

Give an s.c. dose of buprenorphine (0.1 mL) at least 30 min before anesthesia. Sterilize surgical instruments, screws, and screw driver. Transfer sterile tools to 70% ethanol for 60 s and then place tools on a sterile pad to dry. Wipe the stereotaxic apparatus and surrounding area with disinfectant wipes.

3.2.2. Anesthetize Rat

Place rat in the induction chamber. Turn the oxygen on and then isoflurane to 3% with 0.9 L/min flow rate. Wait until the rat is non-responsive to a toe pinch or eye blink test (~3–5 min). Place the rat in a nose cone supplied with a constant flow of isoflurane. Inject the rat with atropine (0.05 mg/kg, s.c.) to decrease pulmonary edema and shave the rat's head before returning it to the induction chamber. Wait another 5–10 min before moving the rat to the stereotaxic device.

3.2.3. Place Rat in Stereotaxic Device

Redirect isoflurane flow from the induction chamber to the Model 1929-B rat gas anesthesia head holder. Slightly pry open the rat's mouth (check to make sure there is no food or debris inside) and place incisors inside the bite bars. Place the ear bars behind the zygomatic arch. Adjust as necessary until the rat's head is centered, does not move side to side, and both ear bars are the same distance from the center. Apply a protective layer of eye ointment over each eye. Maintain the rat temperature around 37°C. Monitor the rat's vitals using a PhysioSuite Monitoring Device attached to one of the rat's feet. Check that the rat is at a surgical plane of anesthesia by pinching the skin between the toes or very gently touching the eyes with a cotton swab. In general, for isoflurane, the rat should have a heart rate less than 350 bpm and respiration rate below 35 bpm when it is at a surgical plane of anesthesia. Proceed when no response to toe or eye stimulation is observed. Sterilize the top of the head by rubbing it with betadine followed by alcohol (make sure neither solution drips down to the eyes). Repeat this process three times.

3.2.4. Expose Skull

Using a scalpel, make an incision from between the eyes to past the ears, holding the skin taut for a smoother cut. Clear the periosteum by pushing it to the sides using a wet cotton swab saturated with saline. Place a pair of curved mosquito forceps on the edge of the cut skin on each side to weigh it down, opening up a bigger clearance to work with on top of the skull. Push the muscle attached to the occipital bone back using a fine-tipped cotton swab soaked with saline immediately caudal to occipital sutures and weigh it down with long bent forceps.

3.2.5. Level Head

Using the Model 1905 alignment tool, adjust the head tilt so that lambda is 4 mm dorsal to bregma. To ensure that the rat is leveled on the sagittal plane, check that the head is leveled 2.5 mm from each side of the sagittal suture. Attach the Model 1915 centering scope and use the positioning knobs to align bregma with the scope crosshair and zero at bregma. Set X-, Y-, and Z-axis digital readouts to zero. Measure coordinates for lambda. Use a pencil or fine-tipped marker to mark the location of the nucleus of the solitary tract (NTS) (AP: −15.3 mm, ML: −1.8 mm).

3.2.6. Drill Pilot and Implant Holes

Using a 0.75-mm drill bit, drill one hole behind the lambdoid suture on the contralateral side of the implant site, and four holes between the lambdoid and coronal sutures (two on each side of and equidistant to the sagittal suture) (Fig.). Drill deep enough (but not through the skull) to anchor the self-tapping screws and then use a screwdriver to manu-

ally lower the ground screw until it touches the cerebral spinal fluid. Note: if using flat-head screws, drill through the skull but do not puncture the brain. For the optrode implant, drill a 2-mm hole through the skull where the NTS was marked taking care not to damage the brain.

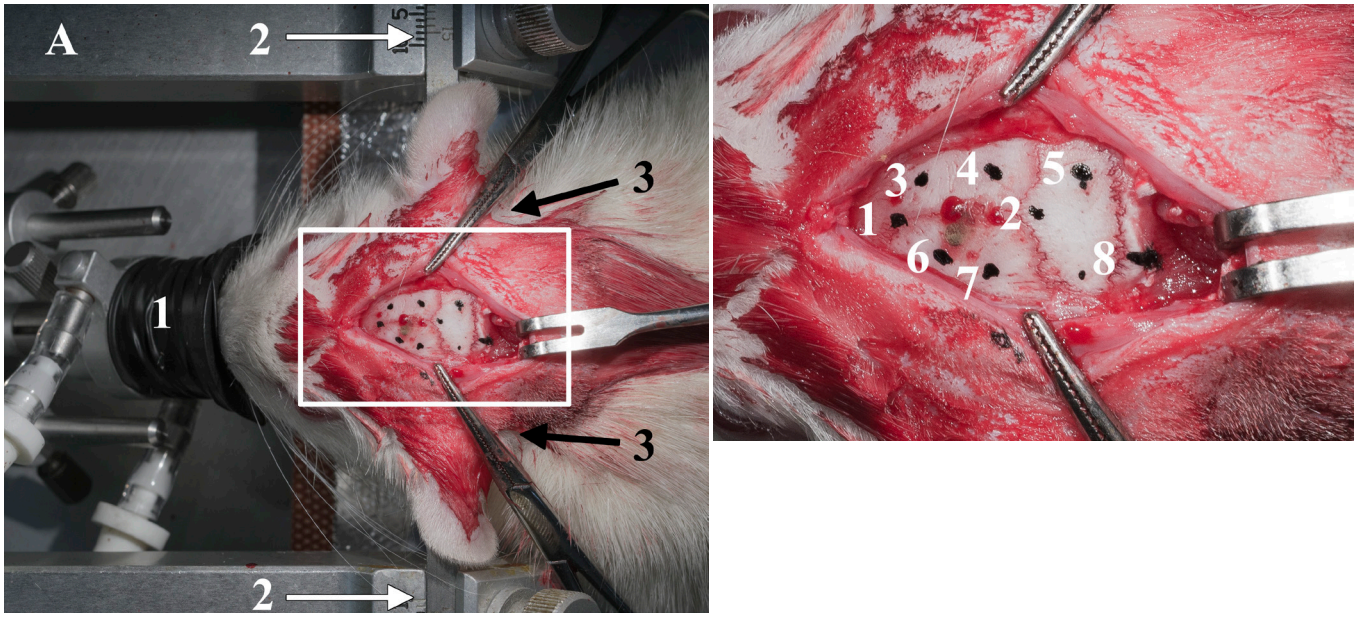


Fig. Rat in the Model 1900 Stereotaxic Instrument. A. The rat's nose is placed in the Model 1929-B rat gas anesthesia head holder (1). The head is held in position by ear bars (2) resting behind the zygomatic arch. The skin on either side of the incision area is held away with mosquito forceps (3). B. Enlarged image of the incision area with dots indicating (1) bregma, (2) lambda, (3–7) bone screws, and (8) NTS.

### 3.2.7. Clear Dura

Clear away debris in the NTS hole and cut through the dura mater using a micro-dissecting knife. Alternatively, a 26-G needle with the tip slightly bent can be used.

### 3.2.8. Implantation Preparation

Connect the amplifier to a pin on the optrode and turn on the oscilloscope, amplifier, and speaker. Note: the speaker volume should be set at zero until the implant is in the brain to reduce electrical artifact. Attach the optrode to the Model 1970 electrode holder and align it to bregma by placing the optrode ~1 mm above bregma, making sure the optrode does not touch the skull as it may bend and damage the wires. Set the coordinates in the digital readout to zero. Move the holder with the optrode to AP: −15.3 mm and ML: −1.8 mm just above the NTS hole.

### 3.2.9. Implant Optrode

Lower the optrode and set the Z-axis to zero when the implant first touches the brain surface. Continue lowering the implant at a rate of 1 mm/5 min, noting the activity as the implant gets deeper into the brain. Around Z= −5.3 mm, start testing for taste responses by moistening the tongue with a cotton swab soaked with taste stimulus. Rinse with artificial saliva after the tastant is applied. Taste responses are usually found between −5.4 mm and −6.1 mm dorsal-ventral. Once a taste response is found, move the optrode ~30

μm dorsal to compensate for any brain swelling. Cover the hole with sterile petroleum jelly or bone wax and place dental acrylic around the base of the optrode to secure the implant position, making sure not to cement the electrode holder with the implant. After the dental cement has hardened, wrap the ground wire around the skull screws and cement around the implant and skull screws to secure it to the skull. Wait until the cement hardens before releasing the implant from the electrode holder.

3.2.10. Rat Recovery

After implantation, supply only oxygen and apply Neosporin around the incision site. When the rat starts to gain consciousness, remove the rat from the stereotaxic device and turn off the oxygen. Once the rat wakes up, provide postoperative care including buprenorphine, gentamicin, and warmed lactated ringers (5–10 mL, s.c.). Provide wet chow in a small bowl for easy food access.

## References


1. **Piccolino M** (1997) Luigi Galvani and rat electricity: two centuries after the foundation of electrophysiology. *Trends Neurosci* 20(10):443–448.

2. **Plank G, Zhou L**, **Greenstein JL**, **Cortassa S**, **Winslow RL**, **O'Rourke B**, **Trayanova NA** (2008) From mitochondrial ion channels to arrhythmias in the heart: computational techniques to bridge the spatio-temporal scales. *Philos Trans A Math Phys End Sci* 366(1879):3381–3409.

3. **Delcour AH** (2013) Electrophysiology of Bacteria. *Annu Rev Microbiol* 67:179–197.

4. **Lin D, Chen J, Lin H, Chen W** (2015) Application of visual electrophysiology for the diagnosis and treatment of cataracts. *Eye Sci* 30(4):190–197.

5. **Uhlhass PJ, Singer W** (2015) Oscillations and neuronal dynamics in schizophrenia: the search for basic symptoms and translational opportunities. *Biol Psychiatry* 77(12):1001–1009.

6. **Zhang F, Gradinaru V, Adamantidis AR, Durand R, Airan RD, de Lecea L, Deisseroth K** (2010) Optogenetic interrogation of neural circuits: technology for probing mammalian brain structures. *Nat Protoc* 5(3):439–456.

7. **Deisseroth K** (2014) Circuit dynamics of adaptive and maladaptive behavior. *Nature* 505(7483):309–317.

## Editor's Column

This issue of the Kopf *Carrier* was written by Joshua Simmons, Ph.D. and Olga Escanilla, MS, a postdoctoral student and Ph.D. candidate respectively. It details the techniques for implanting a viral marker and optrode electrodes into the rat brainstem to evaluate gustatory information processing. The technique utilizes the Kopf model 1900 stereotactic system that provides the most precise and reproducible stereotactic placements available in any stereotaxic instrument. This article provides a detailed step-by-step procedure.

The Society for Neuroscience Convention is just a few months away. We hope to see many of you in Washington in November and invite you to stop by the Kopf Instrument booth to say hi and see the wide variety of stereotaxic and related instruments available from the company.

Almost exactly 36 years ago, in the October 1981 issue of the *Carrier*, the late David Kopf, president of David Kopf Instruments, introduced me as the new Editor of the Carrier. That was issue #8 of the newsletter. The present issue is #91. After this long tenure in the job, I feel that it is time that I retire as Carrier editor and let others take up the task. I will, however, continue to be a consultant with the Kopf Instrument Company for the foreseeable future. The Carrier will continue to bring you useful and interesting information relevant to the Neuroscience community, and about the company itself, under the able leadership of Dawn Gelsinger, Vice President Sales/Operations. I have known Dawn as well as Carol Kopf, company President, for many years and know that the publication is in good hands. It has certainly been my privilege and great good fortune to be associated with this wonderful company for so long, and to be able to continue to be of service.

Michael M. Patterson, Ph.D.
Science Editor (retired)
David Kopf Instruments
drmikep1@me.com

## From the Desk of Carol Kopf

I would like to take this opportunity to thank Michael Patterson, Ph.D. for his many years of service to David Kopf Instruments.

He has not only served as Science Editor for the Kopf *Carrier* for most of its existence, but has assisted our sales team in the booth at Society of Neuroscience meetings. He has been an invaluable advisor and good friend to David Kopf, myself and our staff. We particularly recognize and appreciate his efforts in setting up the SFN David Kopf Lecture on Neuroethics.

At Kopf Instruments we value long-term relationships with our customers, employees, sales representatives, advisors and vendors. They are what makes being in business for 60+ years such a pleasure.

We celebrate Mike Patterson's involvement and look forward to continuing our connection with him and his lovely wife Jan.

Carol Kopf
President
David Kopf Instruments