KOPF®
CARRIER
October, 1991
PUBLISHED BY DAVID KOPF INSTRUMENTS • TUJUNGA, CALIFORNIA • NO 29

Techniques Used for Neural Recording From Awake, Behaving or Anesthetized Animals Part 1

Thomas J. Gould, B.S.
Lonnie L. Sears, M.A.
Joseph E. Steinmetz, Ph.D.

Department of Psychology
And Program in Neural Science
Indiana University
Bloomington, IN 47405

Thomas Gould and Lonnie Sears are senior psychology/neuroscience graduate students in Dr. Steinmetz's Neurobiology of Vertebrate Learning Laboratory at Indiana University. Mr. Gould received a B.S. in psychology from the University of Wisconsin in 1988. Mr. Sears earned a BA. in psychology from Goshen College in 1984 and received an MA. in psychology from Western Kentucky University in 1986. Dr. Steinmetz studied under Dr. Michael M. Patterson and received a Ph.D. in physiological psychology in 1983 from Ohio University. After receiving postdoctoral training in Dr. Richard F. Thompson's laboratory at Stanford University, Dr. Steinmetz joined the faculty at Indiana University in 1987 where he is currently an Associate Professor of Psychology and Neural Science.

Introduction

Over the past several years, the design of stereo-taxic equipment, electrodes, neural recording equipment, and computer-based experimental control devices have improved to the point that it is now relatively easy to monitor neuronal activity from both anesthetized and awake animals. In part due to these technological advances, a great deal of data have been collected in recent years from both invertebrate and vertebrate preparations concerning the neural substrates of a variety of phenomena including sensation, movement, simple learning and memory, emo-tions, and cognition. For example, in the area of learning and memory, intracellular techniques have been used to study behavioral plasticity in invertebrates like Aplysia (e.g., Kandel & Schwartz, 1982) and Hermissenda (Parley & Alkon, 1982) while extracellular recording has provided insight into the neuronal circuitry, activity, and plasticity associated with a variety of mammalian preparations such as eyelid conditioning (e.g., Thompson, 1986), acoustic startle responding (Davis, 1986), and hippocampal LTP (Grover & Teyler, 1990). Our laboratory has been involved in studying the neural bases of two behavioral paradigms, classical eyelid conditioning in rabbits (e.g., Sears & Steinmetz, 1991; and see Gormezano, Kehoe & Marshall, 1983 for a review) and appetitive and aversive signalled bar-pressing in rats (Logue, Miller & Steinmetz, in press). We use a variety of experimental techniques in these studies including extracellular multiple- and single-unit recording, brain mi-crostimulation, lesions, pharmacology and anatomical methods. Detailed here are many of these techniques. In this issue of the Carrier, electrode manufacture, surgical techniques, and electrode and cannula implantation techniques are given. Recording and histo-logical techniques will appear in the next *Carrier*.

Electrodes

All multiple-unit recordings and some single-unit recordings are made with electrodes that we manufacture. We use commercially available electrodes for some single-unit recording applications. Multiple unit recording electrodes are made from 00, stainless steel, insect pins (Carolina Biological Supply, Burlington, NC) that are insulated with plastic resin (e.g., Epoxy-lite #6001-M electrode insulator, Epoxylite Corp., Westerville, OH). The insect pins are prepared for insulation by clipping off their nylon heads and inserting the clipped ends into the flat surface of a 1" cork that has a long 1/4" bolt protruding from the opposite side. Care is taken to align the pins perpendicular to the cork surface making sure that they do not touch each other. Insulation is placed on the electrodes by inverting the cork so that the pin points are oriented downward and slowly dipping the pins into a wide-mouth jar of Epoxylite. At least two methods for dipping the electrodes can be used; (1) The cork's bolt can be clamped in a Kopf electrode manipulator mounted on a stereotaxic frame and the cork assembly lowered in and then raised out of the Epoxylite, or (2) A slow, reversible motor can be fitted with a clamp assembly that holds the cork's bolt to lower and raise the pins. With either method, care should be

(Continued on page 2, Col. 2)

Editor's Column

Here in Ohio we are in the middle of a bad drought. The fanners are going to lose much of their crops and we don't have to mow the lawns as frequently. On the good side, we have had a lot of sunny, bright days. We are not in as bad shape as Southern California where there is water rationing and very tight restrictions on lawn watering. We hope the rain comes soon to both places.

This issue of the Carrier is the first of two parts with a somewhat different slant. The article by Gould, Sears and Steinmetz presents not a single technique, but an experimental strategy made up of several techniques. The rabbit classical conditioning preparation was developed by I. Gormezano in the early 1960s and has become one of the most widely used conditioning preparations for the study of conditioning and brain function. The overview of the methods these investigators use in their approach to their studies should be of wide interest to not only those using the rabbit, but to many others involved in similar studies with other preparations. We hope you enjoy the articles.

Late summer is state fair time. The Ohio State Fair is one of the largest in the country and is going on now. I have heard several times on the news that the animal rights groups have a booth at the Ohio State Fair at which people can learn the terrible things going on in research with animals. It certainly is true that we must continue and increase our efforts to educate the public about the benefits of research with animals. Such developments as the Alzheimer Mouse model just announced should catch some attention and make it easier to convince the public of the vital importance of such research. Do what you can to help educate the public about the value of the information gained through research and how it directly helps every one of us, and the very animal populations which are used to supply the information.

If you would like to contribute an article to the Carrier, contact me for details at the address below.

Michael M. Patterson, Ph.D.
Science Editor
College of Osteopathic Medicine
Ohio University
Athens, OH 45701
Phone-(614) 593-2337 Fax-(614) 593-9180

taken to maintain a slow, steady rate when dipping and raising electrodes and to prevent the cork from making contact with the resin. We have found that even coats of insulation are produced when the pins are lowered slowly then immediately, but slowly, raised (i.e., not allowed to remain stationary in the Epoxylite). After each dip, the electrodes are baked in an oven for 3-12 hr at 99° C. Depending on the age of the Epoxylite, rate of dipping, and amount of time the electrodes are baked between dips, 12-20 dips are required to adequately coat the electrodes.

We have also manufactured tungsten single-unit recording electrodes. Lengths of tungsten wire (25 urn diameter) are cut and either etched in an electrolyte solution (e.g., potassium chloride) or flame-formed to a point using an acetylene torch. The pointed electrodes are then insulated as described above. We have also purchased single-unit recording electrodes from two sources (Frederick Haer, Co. Brunswick, ME and A-M Systems, Inc., Everett, WA). These commercially obtained electrodes come in a variety of diameters, tip profiles and impedances.

After insulating the multiple-unit or single-unit electrodes, they are prepared for specific uses. Typically, we determine the depth of the structure to be recorded from and remove excess insulation at the top (blunt-end) of the electrode. For electrodes that area? ^ be chronically-implanted, we wrap, then soldei~sr *" length of 30 gauge stainless steel wire to the uninsulated, top portion of the electrode. We next remove the insulation at the tip of the electrode using a high-voltage pulse generator described by Ciancone and Rebec (1989). Briefly, this device uses an ignition coil to produce a spark which can be arced between a fully insulated electrode and a counter electrode (typically a flat bolt). Under a microscope, the electrode is positioned close to the bolt, a switch is closed to arc the spark and in the process, insulation is removed. The impedance of the electrode tip is determined by the number of sparks thai are applied. For impedances in the range of 50 kHz to 5MHz, a BAK Model IMP-1 electrode impedance tester (BAK Electronics, Inc., Germantown, MD) can be intermittently used to monitor changes in tip impedance. Low impedance electrodes with rather uniform tip size, such as those used to deliver brain lesions or stimulation, can be created by scraping insulation from the tip with a #11 scalpel blade while viewing the electrode through a dissecting microscope equipped with a calibrated graticule in one eye piece. We have also used dental acrylic, applied to the uninsulated portion of the electrode shaft, to cement several electrodes together to form recording or stimulating assemblies.

Surgery

Anesthesia, Aseptic procedures are followed for all chronic surgeries involving rabbits or rats. Rabbits are anesthetized with a mixture of ketamine HC1 (Keta-set, Aveco Co., Inc., Fort Dodge Iowa) and xylazine

(Continued on page 3, col.1)

(Rompun, Mobay Corp., Shawnee, KS). Anesthesia is induced by first injecting xylazine (6 mg/kg, s.c.), waiting 15 min then injecting ketamine (60 mg/kg, i.m.). A mixture of the xylazine and ketamine is injected i.m. once every 45 min to maintain a surgical level of anesthesia. Rats are anesthetized with cholor-opent (a solution containing 0.588 g of pentobarbital and 2.55 g of chloral hydrate) that is delivered i.p. (0.33 mg/kg). A surgical level of anesthesia is maintained by giving additional i.p. injections once every 60-90 min.

Sterilization. Most items used in surgery are sterilized in a steam autoclave beforehand. However, we use a cold sterilization method to disinfect several other items used during surgery. We have found Instrument Germicide (Lehn & Fink, Toledo, OH) and Cidex (Johnson & Johnson, Arlington, TX), both which contain a rust-inhibitor, to be very effective. Stainless steel screws, electrodes, drill bits, miniature screw drivers, wire cutters/crimpers, stereotaxic alignment tools, ground wires, micromanipulator bases, and plug assemblies are all placed in these sterilizing solutions for at least 15 min. We have had problems, however, sterilizing some Kopf electrode holders. The Model 1774 holder, which has an all-metal construction, can be fitted with the electrodes before surgery and placed in the sterilizing solution. Models 1770 and 1771 have acrylic blocks against which the electrodes are clamped and can be damaged by the solutions (i.e., the acrylic blocks disintegrate and sometimes loosen from the shaft). We therefore recommend that these holders not be used when cold sterilization procedures are used. We do not sterilize the electrode manipulators or the surgery drill but rather pre-sterilize a generous supply of large, gauze sponges through which we handle non-sterile items during surgery.

Stereotaxic Placement. Almost all chronic implants that we perform require stereotaxic placement of the electrodes. For rat surgeries, we use a Kopf Model 1430 stereotaxic frame and associated electrode manipulators. For these surgeries, the rats' heads are fixed using a rat tooth bar, nose clamp and ear bars (Model 1220). The heads are brought into stereotaxic plane by moving the tooth bar assembly and by centering the ear bars. Stereotaxic atlases by Pellegrino and Cushman (1967) and Paxinos and Watson (1986) have produced good results. For rabbit surgeries, we also use the Model 1430 stereotaxic frame and associated manipulators. The rabbits' heads are held with either a custom-made head-holder that clamps over the muzzle region or the Kopf Model 1240 rabbit adaptor that secures the head via zygoma clamps, adjustable tooth bar and nose clamp. All stereotaxic procedures are conducted in a stereotaxic plane that positions the bregma skull landmark 1.5 mm above the lambda landmark. A Kopf rabbit alignment tool (Model 1244) is used to find this position.

Chronic Electrode Implants. In rats and rabbits, most of our studies involve chronically implanting multiple-unit recording electrodes, microstimulation electrodes, or lesion electrodes into specific brain targets (e.g., Sears & Steinmetz, 1991). Holes through which the electrodes will be lowered are first drilled into the skull using a Dremel Model 232-5 variable speed drill equipped with a size 6 carbide drill bur (Roboz Surgical Co., Inc., Washington D.C.). We also drill 1-3 holes and thread #2-56 stainless steel bolts into them. These bolts serve two purposes. First, they serve as anchor points to secure dental acrylic that is used to cement electrodes into place. Second, we wrap 30 gauge stainless steel wire around the bolts and eventually use the wire as ground points for recording, stimulating or lesioning. The electrode holes are then filled with bone wax, each electrode is secured in a electrode clamp and lowered into the brain with an electrode carrier that is mounted on the stereotaxic frame. We have found that in addition to using stereotaxic calculations, recording activity from the electrodes, while they are lowered, increases our accuracy of hitting the brain targets. During this procedure we place a small Faraday cage around the preparation and connect the electrode leads and ground to a Grass P15 dc amplifier that is placed inside the cage. Output from the P15 is directed to a Tektronix 2055 portable oscilloscope and to a Radio Shack SA-10 Amplifier with speaker that allows audio monitoring of neuronal activity. Once the final position of each electrode has been determined, each electrode is cemented into place with a small amount of dental acrylic (Caulk Grip Cement, Milford, DE). After all electrodes have been positioned, the stainless steel leads from each electrode (that were cemented to the electrode shafts as described above), together with the stainless steel ground leads are routed to gold male Amphenol pins that are inserted into a standard Amphenol strip. Care is taken to make sure that the leads do not touch each other and that the acrylic is not bound to tissue surrounding the skull. After all wires are positioned, dental acrylic is used to completely cover the wires, ground screws, and electrode shafts. If other equipment must be secured to the head stage (such as screws or threaded blocks for holding stimulus delivery or behavioral recording equipment), it can be placed into the acrylic before it sets. The area around the implant is then painted with an iodine-based ointment and the wound is closed with 4-0 nylon suture. All animals receive an i.m. injection of Ambi-pen (0.33 cc) to aid recovery.

Cannula Implants and HRP Injections. We have conducted a few experiments that required the delivery of lidocaine to specific brain regions to produce temporary lesions during behavioral training (e.g., Chapman, Steinmetz, Sears & Thompson, 1990). Stainless steel 22 gauge tubing, cut to the desired length, has been stereotaxically positioned then cemented into place as a cannula for these injections. Between behavioral training sessions, a stylus can be

(Continued on page 4, col. 1)

inserted into the cannula or the top of the cannula can be fitted with a stopper to prevent the brain from being exposed. During behavioral training, pressure injections are made into the brain via a needle from a Hamilton syringe that is inserted into the implanted cannula. We have also attached a recording electrode to the cannula using dental acrylic. This electrode can serve two functions. First, it increases the accuracy of placement as the cannula can be guided into final position on the basis of observing characteristic neuro-nal activity. Second, placement of the electrode allows neuronal activity to be monitored during the application of solutions through the cannula (e.g., the local anesthetic effect of lidocaine could be verified by recording before, during and after its application). In other experiments, we have injected horseradish peroxidase (HRP) during aseptic surgery (Steinmetz & Sengelaub, 1989). After determining stereotaxically where to place the HRP injection, we mounted an HRP-filled Hamilton syringe in a Kopf Model 1772 universal holder and secured the holder in a manipulator. Using the manipulator, the needle was then positioned and slowly lowered to the injection site and, over a 5 min. period, the volume of HRP was slowly pressure-injected (manually or using a slow-speed syringe pump) into the site. After leaving the needle in place for 10-15 min. after the injection, the needle was very slowly withdrawn (to minimize HRP from following the needle out of the injection site). We have made satisfactory injections of wheat germ agglutin conjugated HRP and cholera toxin conjugated HRP using this method. To increase the accuracy of placing the HRP, we have also insulated a Hamilton syringe needle with Epoxylite using a dipping and baking process similar to that described above. During dipping, a wire must be inserted into the needle to prevent it from filling with Epoxylite. After the needle is insulated, the wire is removed and the tip scraped to create a "recording needle". Activity can then be recorded from the needle as it is lowered into position.

Electromyographic Recording Electrodes. Because we often monitor behavior during neuronal recording sessions (e.g., classical eyelid conditioning in rabbits or signalled bar-pressing in rats), it is sometimes necessary to implant electromyographic (EMG) recording electrodes during surgery. Ilie EMG recordings are typically taken via uninsulated 30 gauge stainless steel wires that are implanted into the muscle(s) of interest (e.g., eyelid closure musculature). To implant the EMG wires, we insert a 1", 26 gauge hypodermic needle through a portion of the muscle. The stainless steel wire is then inserted all the way through the needle, the wire is held, and the needle is withdrawn thus leaving the wire imbedded in the muscle. The two ends of the wire are then twisted together to form a rather tight loop and a male Amphenol pin is crimped onto the twisted wire paired. We typically place two wires into each muscle, one to serve as an active lead and the other to serve as a ground or indifferent. A connection to the ground pin in the acrylic head stage assembly can also serve as a reference point for the EMG recordings.

Single-Unit Recording and Acute Preparation. Experiments involving extracellular single unit recording in behaving animals (e.g., White, Rebec & Steinmetz, in press) or single units and population potential recording from acute, anesthetized animals (e.g., Gould, Sears & Steinmetz, 1990) are also conducted in our laboratory. The animals are prepared for chronic single-unit recording in the same manner as described above for multiple -unit sessions with one exception: Instead of chronically implanting electrodes, we implant a micromanipulator base over a wide opening that is created in the skull. We have found that the Narishige MO-99 hydraulic microdrive and head stage system (Medical Systems Corp., Greenvale, NY) works well for these recordings. In this system, the base is cemented onto the skull with dental acrylic and during recording sessions, a lightweight micromanipulator is secured to the base. After surgery, the base is filled with petroleum jelly and capped. An electrode is then mounted in the micromanipulator and slowly lowered into the brain until a single unit has been isolated. The same amplifiers and spike detection equipment that are described below for multiple unit recording sessions are used for the single unit sessions.

Surgical techniques used during chronic implant procedures are also used during acute, non-survival procedures (with no aseptic conditions). Our acute studies typically involve recording stimulation-evoked population potentials or single-unit activity to examine connectivity and electrophysiological properties of neuronal populations. For both types of recordings, we slowly lower the recording electrodes with a Narishige MO-81 stepping hydraulic micro-drive that is held over the preparation with a Kopf universal holder. Output from the electrode is amplified by a de-powered Grass Model PI 5 amplifier then routed to a BP GE-333 Equalizer (Fordham Electronics, Hauppauge, NY) that filters the signal (e.g., 500-5000 Hz for single-units; 100-1000 Hz for population potentials). Output from the equalizer is routed to a Kikusui DSS5040 digital storage scope, to a Haer Model #74-30-1 Audio Analyzer, and to an MI2 M100 signal processing system (Modular Instruments Inc., Southeastern, PA) that is interfaced with an IBM-AT clone computer. For single-unit recording a MI2 window discriminator module is used to select action potentials that exceed a preset threshold. We use software available from MI2 to record, store and analyze population potentials as well as discriminated single units. This same system has also been used to record EEC activity from awake animals as well as population potentials evoked by auditory stimuli or brain stimulation delivered to awake animals.

Part 2 will appear in the next *Carrier*.

(Continued on page 5, col. 1)

REFERENCES

Chapman, P.F., Steinmetz, J.E., Sears, L. L. & **Thompson, R.F.** (1990). Effects of lidocaine injection in the interpositus nucleus and red nucleus on conditioned behavioral and neural responses. *Brain Research*, 537,149-156.

Gancone, M.T. & Rebec, G.V. (1989). A simple device for the reliable production of varnish-insulated, high-impedance tungsten microelectrodes. *Journal of Neuroscience Methods*, 27,77-79.

Davis, M. (1986). Pharmacological and anatomical analysis of fear conditioning using the fear-potentiated startle paradigm. *Behavioral Neuroscience*, 700,814-824.

Parley, J. & Alkon, D. L. (1982). Associative neural and behavioral change in Hermissenda: Consequences of nervous system orientation for light and pairing specificity. *Journal of Neurophysiology*, 48, 785-807.

Gould, T. J., Sears, L. L. & Steinmetz, J. E. (1990). Rabbit cerebellar activity evoked by pontine nuclei or inferior olive stimulation-Potential regions of CS-US convergence during eyelid conditioning. *Neuroscience Abstracts*, 16,763.

Gormezano, I., Kehoe, E.J., & **Marshall, B.S.** (1983). Twenty years of classical conditioning research with the rabbit. In J.M. Sprague & A.N. Epstein (Eds.), *Progress in learning and motivation* (Vol. 10, pp. 197-275). San Diego, CA: Academic Press.

Grover, L. M. & **Teyler, T. J.** (1990). Two components of long-term potentiation induced by different patterns of afferent activation. *Nature, 347*,477-479.

Kandel, E. R., & **Schwartz, J. H.** (1982). Molecular biology of learning: Modulation of transmitter release. *Science, 218*,433-442.

Logue, S. P., Miller, D. P. & **Steinmetz, J. E.** (in press). A signalled bar-pressing preparation for study of the neural bases of appetitive and aversive learning in the rat. *Neuroscience Abstracts.*

Paxinos, G. & **Watson, C.** *The rat brain in stereo-toxic coordinates. Second Edition.* Academic, New York, 1982.

Pelleerino, L. J. & **Cushman, A. J.** *A stereotaxic atlas of the rat brain.* Appleton-Century-Crofts, New York, 1967.

Sears, L. L. & **Steinmetz, J. E.** (1991). Dorsal accessory olive activity diminishes during acquisition of the rabbit classically conditioned eyelid response. *Brain Research, 545*, 114-122.

Steinmetz, J. E. & **Sengelaub, D. R.** (1988). Direct projections from the lateral pontine nucleus to the anterior interpositus nucleus: A potential CS pathway for classical conditioning. *Neuroscience Abstracts, 74*,782.

Thompson, R. F. (1986). The neurobiology of learning and memory. *Science, 233*,941-947.

White, I. M., Rebec, G. V. & **Steinmetz, J. E.** (in press). Conditioning-related single-unit activity in the neostriatum during classical eyelid conditioning in rabbits: Effects of haloperidol. *Neuroscience Abstracts.*

NEW PRODUCT ANNOUNCEMENT

The David Kopf Instrument Company is proud to announce the following new products, which will be on display at the Society for Neuroscience Meeting in New Orleans.

Model 1541 Piglet Adaptor which will fit all 1530 stereotaxic frames

Model 935 Electrode Angle Calibrator for the Model 900 Small Animal Stereotaxic

Newly modified Mouse Adaptor and Head Stabilizer for all frames

The new Model 962 Small Animal Stereotaxic will also be shown