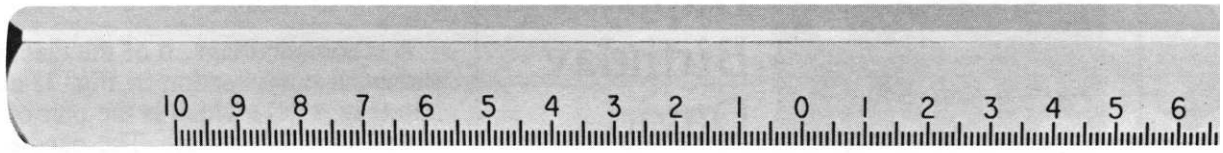
KOPF
CARRIER
September, 1992
PUBLISHED BY DAVID KOPF INSTRUMENTS • TUJUNGA, CALIFORNIA • NO 32



An Easily Fabricated Electrode Holder for Voltammetric and Electrophysiological Recordings from Awake, Behaving Rats

R. Christopher Pierce, B.A.
Zhongrui Wang, M.S.
Byron A Heidenreich, B.S.
Paul E. Langley
George V. Rebec, Ph.D.

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

Christopher Pierce and Zhongrui Wang are senior graduate students in neural science, Byron Heidenreich is a senior graduate student in psychology and Paul Langley is a technician in Dr. Rebec's Preclinical Neuropsychopharmacology Laboratory at Indiana University. Mr. Pierce received a BA. in psychology from the University of Kentucky in 1988. Mrs. Wang received a B.S. in electronic engineering in 1968 and a M.S. in biomedical engineering in 1983 from Tsinghwa University. Mr. Heidenreich received a B.S. in psychology from the University of Illinois (Urba-na-Champaign) in 1983. Mr. Langley has been a technician in Dr. Rebec's lab for the past four years after leaving the University of Missouri School of Medicine where he was employed for 28 years. Dr. Rebec studied under Dr. Phillip M. Groves and received a PhD. in biopsycholo-gy from the University of Colorado in 1975. After receiving post-doctoral training in Dr. David S. Segal's laboratory at the University of California, San Diego, Dr. Rebec joined the Indiana University faculty in 1977 where he is currently Professor of Psychology and Neural Science, and Director of the Program in Neural Science.

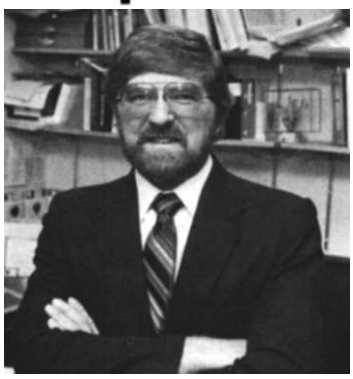
INTRODUCTION

Recent technological advances have allowed investigators to apply single-unit electrophysiolo-gy (Lemon, 1988) and in vivo voltammetry (Adams, 1990) to awake, behaving rats. This relatively new experimental approach permits concurrent evaluation of drug- or learning-induced changes in neuronal and neurochemical activity with ongoing behavioral events. Adapting a recording technique to freely moving rodents, however, creates a number of problems. First and foremost, a device is needed to raise and lower electrodes through the brain, without rotating them and without the aid of the calibrated arm of a stereotaxic instrument. Ideally, such a device should be as small and lightweight as possible to allow the animal unrestricted movement. Also, it should be easy to install on the head to allow the electrode to be implanted on the day of the experiment to avoid potential degradation of the electrode from chronic exposure to the brain milieu. We have devised such a device in our laboratory for voltammetric and single-unit recordings from the neostriatum of freely-moving rats. The entire system is inexpensive and relatively easy to fabricate; the basic pans are readily available in most neuroscience laboratories.

MATERIALS

The design of our electrode holder is based on the luer-lock coupling between syringes and needles. Thus, a plastic 1-ml syringe and corresponding needle (Becton, Dickinson & Co.) are required. Additional supplies include a few cm of 17 gauge tubing, brass rods of between 3 and 8 mm diameter, a brass screw (4-80 threads, 2 mm diameter), 10 and 14 mm diameter nylon rods, two 2-56 3/16" brass screws, and two small Augat socket terminals (#LSG-1AG2-1). (These materials were either obtained locally or through Small Parts Inc.).

(Continued on page 2, Col. 2)



Happy Birthday David Kopf

On August 9, 1992, David Kopf celebrated his 65th birthday. Not only that, but it really was his birthday. And despite what he says, it really was his 65th. As they usually do, David and his wife Carol, did the celebration up in grand style. They had an all afternoon pig roast and general party in the Kopf Instrument parking lot. Almost 300 people attended the bash, including David's mother and many of his family. A delightful Dixieland band played in the tent (there to keep out the sun, not the rain) for the guests and there was entertainment for the kids of all ages.

One of David's current main avocations is race cars, and driving them. On one side of the lot is a large building in which were displayed his current crop of 8 or 9 new and vintage race cars, some of his collection of calliopies (remember the Mighty Ruth?) and other varied things like trophies and memorabilia. The guests could stroll through the area looking at the cars, listen to David shyly recounting his exploits on the track, and admire the beautiful antiques on display. Indeed, many of the guests were friends from the racing circuit.

David played with the band, enjoyed hearing the reminiscences of friends and relatives, and eventually opened a lot of both serious and not-so-serious gifts. The employees of David Kopf Instruments even gave the Boss a replica of one of the race tracks made out of silver dollars.

It was a wonderful day honoring David on his birthday. Please stop by the Kopf Instrument Booth at the Society for Neuroscience Meeting to congratulate David on his 65th. He will be glad to tell you about his racing exploits, show you some racing pictures, or talk with you about stereotaxic instruments or pipette pullers.

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

FABRICATION

A schematic diagram of the electrode holder is shown in cross-section in Fig. 1, along with the hub (Fig. 1, #1) which is the part of a needle that mates with the syringe. The needle has been removed and the remaining hub cut down to 5 mm creating a small plastic tube with angled sides and a lip on one end. During surgery, this hub is centered over a hole that has been drilled through the skull overlying the structure of interest (for our purposes, the rat neostriatum: 1 mm anterior and 2.5 mm lateral to bregma) and cemented in place with dental acrylic. We have found, particularly for experiments involving multiple recording sessions or chronic drug treatment, that setting two 0-80 machine screws into the skull near the hub and covering them with the dental acrylic allows the hub and cement to remain in place for as long as several months.

The hub mates with a standard 1-ml syringe via the luer-lock coupling. Thus, we build our electrode holder around a 1 ml syringe, cut down to 2.5 cm in length with 10-32 threads cut into the larger opening of the syringe (Fig. 1, #2). A brass collar (optional) is placed on the threaded end of the syringe for structural support. A guide cannu-la has been included in our design (Fig. 1, #3) in order to breach the dura. (We use carbon fiber electrodes for voltammetric recordings which are destroyed if the dura is not punctured.). The guide cannula is a 13-mm length of 17-gauge tubing one end of which is beveled at a 45° angle. A small brass collar must be machined in order for the guide cannula to fit securely into the syringe tip. We use an 8-mm brass tube with an inside diameter slightly larger than the outside diameter of the 17-gauge cannula (i.e .058") with a slight angle cut into the outer wall of the brass tube (from 1.8 mm at the proximal to 2.0 mm at the distal end). This brass collar is soldered over the unbeveled end of the cannula, and the whole piece is placed in the small diameter end of the syringe with the beveled end of the cannula extending 5 mm from the syringe. The slight angle of the brass collar allows this piece to fit snugly into the small end of the syringe; that is, this piece is held in place by friction between the brass collar and the plastic syringe. When the electrode holder mates with the hub, the beveled end of the cannula extends 2.5 mm beyond the edge of the hub cemented to the skull (approximately 1.5 mm into the brain).

The electrode carriage (pieces #4 and 5) and

(Continued on page 3, col.1)

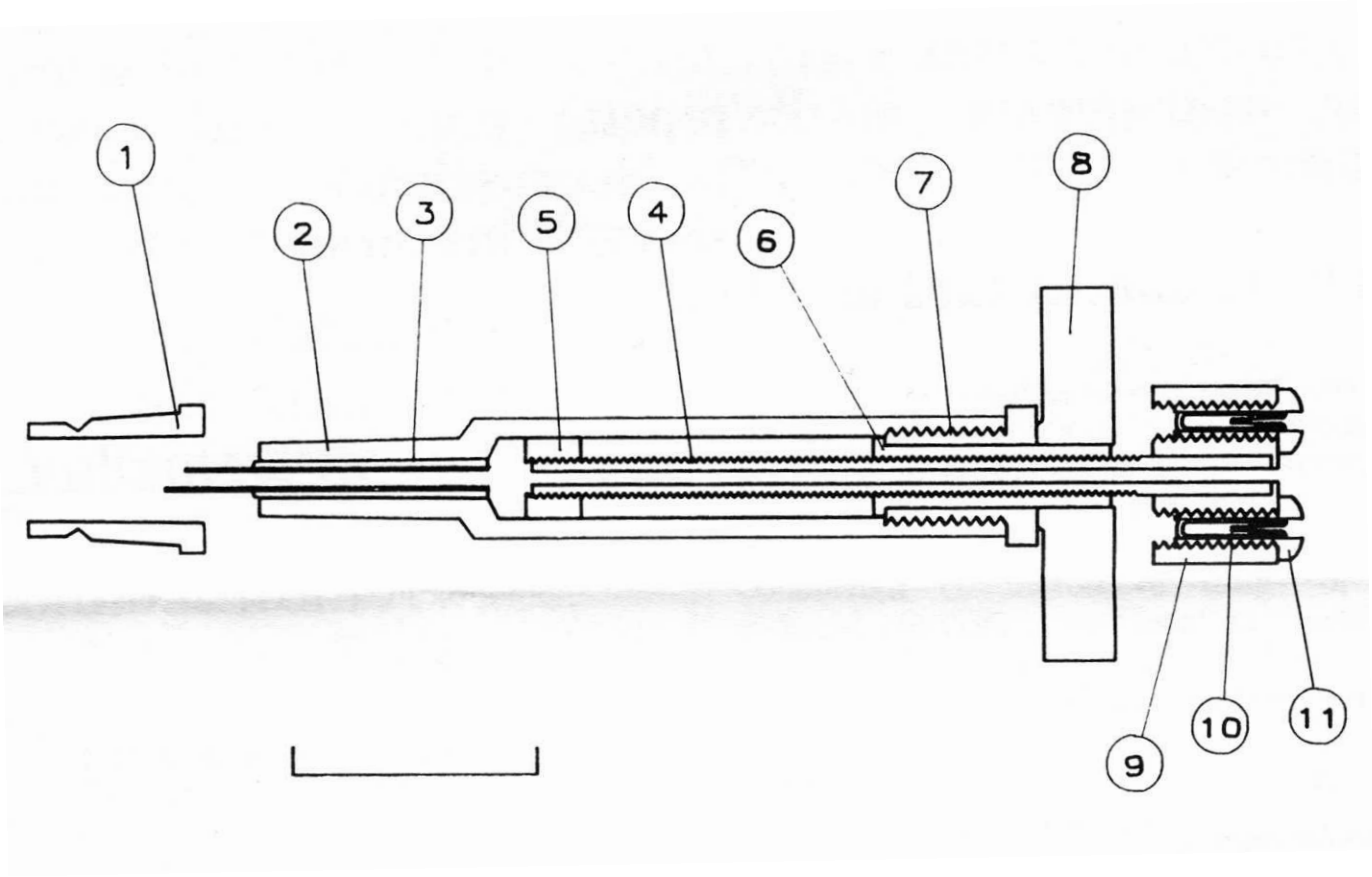


Figure 1. A cut-away diagram of the skull hub (#1), electrode holder (#2-3), and microdrive (#4-8), as described in the text. The top of the microdrive assembly (#9-11) serves as the connecting point for both the metal lead wires from the working and reference electrodes and the coaxial cables that connect the electrodes to recording equipment via a swivel. Calibration bar: 10 mm.

microdrive assembly (pieces #6-8) allow the electrode to be raised and lowered into and out of the electrode holder and through the brain. The electrode carriage is a 2-mm diameter screw (4-80 threads) 3 cm in length with a 1-mm diameter hole drilled through the center (Fig. 1, #4). Thus, the screw (piece #4) becomes a tube with a 1-mm inside diameter and a 2-mm outside diameter. Threaded onto the end of this screw (and glued in place) is a 3.8 mm brass cube with a 1-mm hole drilled through the center and 4-80 threads tapped inside the hole (Fig. 1, #5). (The cube eventually will fit snugly inside the syringe thus preventing the rotation of piece #4). The microdrive assembly, which also threads onto piece #4, is itself composed of three separate pieces (Fig. 1, #6-8), Piece #6 is a brass tube 12 mm in length with an inside diameter of 2 mm and 4-80 threads tapped inside of it (allowing piece #6 to be screwed onto piece #4). The outside diameter of this tube is 3 mm and smooth except for a 1-mm lip on one end of the tube, which is 4 mm in diameter. Piece #6 is screwed onto piece #4 with the lip end toward the 3.8 mm cube. Piece #7 is a brass tube 7 mm in length with an inside diameter of 3 mm and an outside diameter of 4.5 mm. The inside of this tube is smooth, but the outside is cut with 10-32 treads except for a 1-mm Up at one end which is 8 mm in diameter. Piece #7 is placed over piece #6 with two lips on opposite ends. Piece #7 should move freely around piece #6. Piece #8 is a 4 mm tall disk cut from the end of a 14-mm diameter nylon rod with a 2.8 mm hole cut through the center. Piece #8 is placed over the end of piece #4 (sandwiching piece #7 between the lip of piece #6 and piece #8) and held in place by friction. Piece #7 should turn

(Continued on page 4, col. 1)

freely between pieces #6 and #8 and the whole assembly should move up and down piece #4 when piece #8 is turned. Piece #7 is designed to screw into the large end of the syringe. Thus, when the electrode holder is fully assembled and piece #8 is turned, the electrode carriage (pieces #4 and #5) will move into and out of the syringe. The last piece of the electrode holder is a 5 mm long section of 10-mm diameter nylon rod with a 2-mm hole cut through the middle and 4-80 threads tapped inside this hole (Fig. 1, #9). Two additional holes are drilled through this nylon piece on either side of the center hole, and threads are cut into these holes to accept 2-56 brass screws (Fig. 1, #11). The nylon piece is screwed onto the end of the 3-cm tube and glued in place. A hole is drilled through each of the 2-56 screws to accept a small Augat socket terminal (Fig. 1, #10). The Augat terminals are pressed into the center of the screws (female opening toward the head of the screw) and held in place by friction. These screws serve as connecting points for the working and electrode reference electrodes. Electrical connectivity is maintained because the metal leads from electrode and ground can be held under these brass screws, which also connect to coaxial cables that have been fitted with Augat male terminals on one end (the other end of the coaxial cables connect to a swivel that allows free movement of the animal).

Fully assembled, our electrode holder also is suitable for experiments in which it is desirable to infuse drugs directly into the brain. The 1-mm hole in the middle of the electrode holder provides ample space for electrodes (voltammetric or electrophysiological) as well as a 33-gauge infusion cannula, which allows drugs to be administered very close to the active surface of our electrodes (i.e. 100 to 500 mm away). In fact, an infusion cannula, when bent at a 9Qo angle and screwed under one of the 2-56 brass screws, serves as the reference electrode for our voltammetric recordings

We have been using the electrode holder successfully for several years (Pierce and Rebec, 1990; Tschanz et al., 1991; Wang and Rebec, in press). Fig. 2 shows an oscilloscope trace of single unit activity (top) and a sample voltammo-gram (bottom) obtained during infusions of 30 mg/ml D-amphetamine (10 ml/hr) directly into the neostriatum of awake, behaving rats. Approximately 15 min. following infusion onset, typical amphetamine-induced behaviors, including sniffing, head bobbing and locomotion, were observed. These behaviors persisted for the duration of the voltammetric and electrophysiological recording periods.

CONCLUDING REMARKS

The electrode holder that we have described is inexpensive, easy to make, and permits voltammetric and electrophysiological recordings to be made in conscious, behaving rats. Besides the obvious benefit of correlating ongoing behavior and neuronal activity, this type of experiment eliminates the need to consider anesthetic influences on brain activity. We believe that current and future methodological advances such as our electrode holder will foster a better understanding of brain-behavior relationships.

REFERENCES

Adams, R.N. (1990). In vivo electrochemical measurements in the CNS. *Progress in Neurobiology,35*, 297-311.

Lemon, R. (1984). *Methods for neuronal recordings in conscious animals*. New York: Wi-ley Press.

Pierce, R.C. & Rebec, G.V. (1990). Stimulation of Both Dl and D2 dopamine receptors increases behavioral activation and ascorbate release in the neostriatum of freely moving rats. *European Journal of Pharmacology, 191*, 295-302.

Tschanz, J.T., Haracz, J.L., Griffith, K.E. & **Rebec, G.V.** (1991). Bilateral cortical ablations attenuate amphetamine-induced excitations of neostri-atal motor-related neurons in freely moving rats. *Neuroscience Letters, 134,* 127 -130.

Wang, Z. & **Rebec, G.V.** (in press). Neuronal and behavioral correlates of intrastriatal infusions of amphetamine in freely moving animals. *Society for Neuroscience Abstracts.*

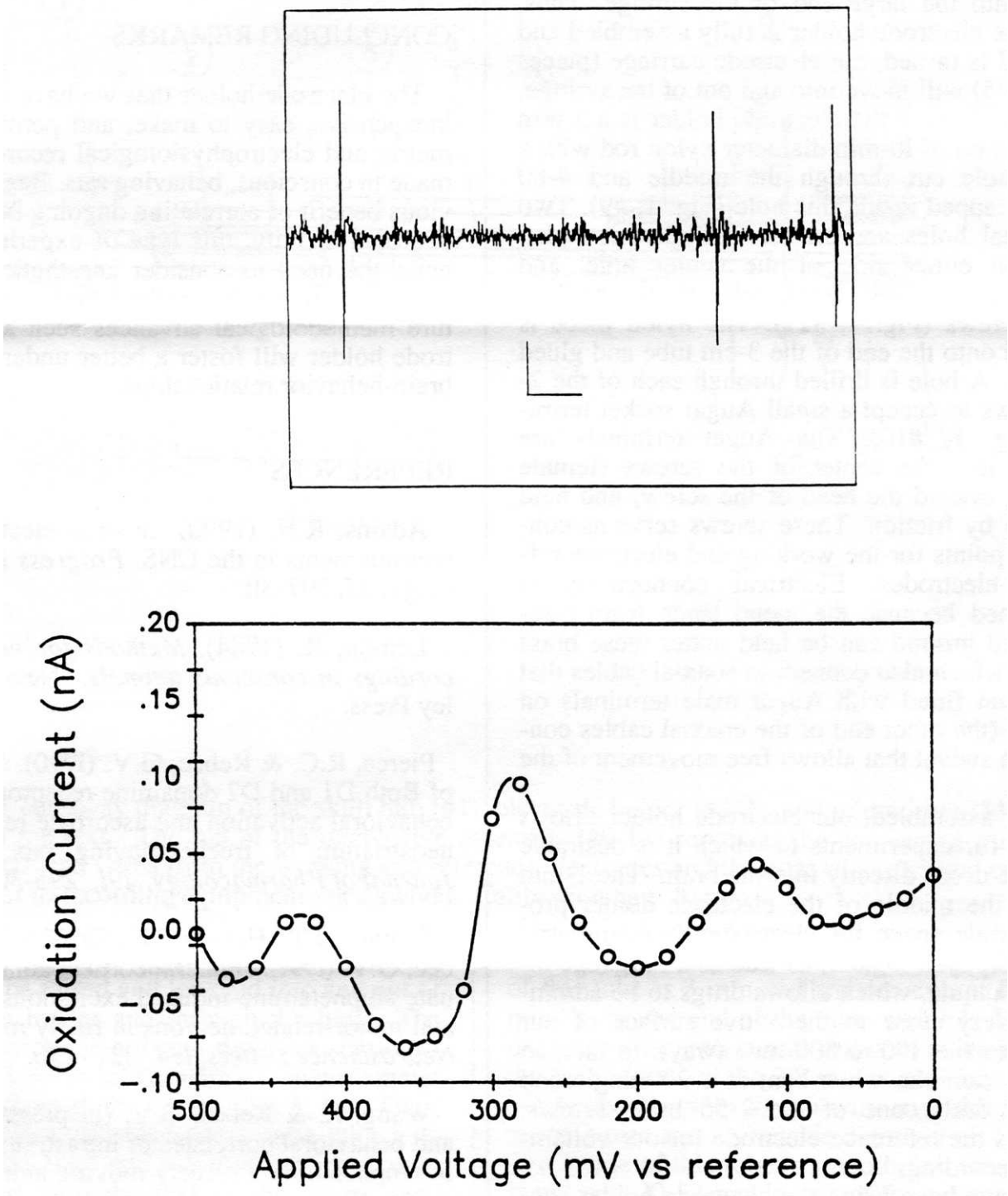


Figure 2. Single unit activity (top) and a representative voltammogram (bottom) obtained during neo-striatal amphetamine infusion (30 mg/ml; 10 ml/hr). Approximately 15 min following infusion onset, typical amphetamine-induced behaviors, including sniffing, head bobbing and locomotion, were observed. These behaviors persisted for the duration of the voltammetric and electrophysiological recording periods. Calibration bar (top): 40 mV (vertical), 10 mS (horizontal).