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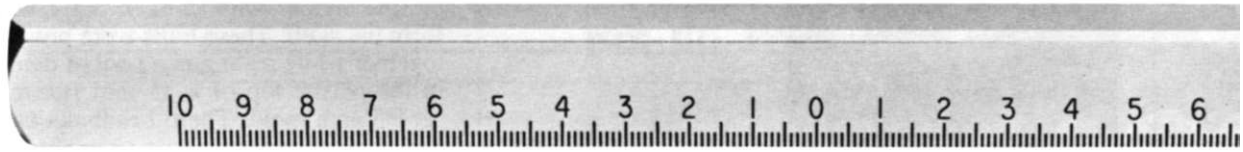

MULTICHANNEL EXTRACELLULAR RECORDING TECHNIQUES

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INTRODUCTION

Classical extracellular single cell recording has relied greatly on the use of single microelectrodes and simple window discriminators. However, with developments in the analysis of multichannel recordings, multichannel electrodes are becoming easier to use for examining simultaneous local and distributed information processing. The discrimination of waveshapes, which characterize individual neurons, has also become much more sophisticated. In this review we discuss some of the techniques we use which incorporate these advances.

ELECTRODES

Two types of multichannel electrodes have been used in our laboratory. The first, is modeled after Kubie's (1984) design, and has successfully recorded single units form the rabbit hippocampus (Thompson et al. 1990). It consists of a bundle of five twisted pairs of formvar insulated nichrome wires (25 um). The individual wires are attached to the pins of a connector (Augat # 8058-1G34) and the bundle is supported by a 28 gauge stainless steel guide tube. The guide tube also serves as the reference electrode. The connector is affixed with dental cement within the center of a tripod formed by three 1-72 x 3/4" machine screws. The head of the screws are flush with the top of the Augat connector, and the tip of the screws are partially driven into standoffs (pieces of strip connector that are tapped for the screw).

The other electrode is commercially available and relatively inexpensive (NB Labs, Denison, TX). It consists of an array of Teflon coated 50 um stainless steel wires which are attached to a strip connector. We have used the 2 x 4 array, although other arrays and bundles are available. Most configurations include a ground wire and stimulation wire. Before surgery a tripod is constructed for this electrode as for the Kubie style electrode.

During surgery the electrodes are held by a mating plug which is secured by an electrode holder. If the electrode is to be placed only by stereotaxic coordinates, a mating plug with a screw threaded through the center is used to support the connector. If the electrode is to be advanced under physiological control a right angle adapter securely positions the electrode assembly while a cable is simultaneously connected between the electrode and the recording equipment.

SURGERY

Surgeries are done with sterile technique using ketamine and xylazine as an anesthetic for the rabbit. A stereotaxic with zygomatic arch clamps is used to secure and position the skull. After the skull is exposed and cleaned six stainless steel self tapping screws (#2 x 1/4", Small Parts, Miami Lakes, Ft.) are implanted. These screws serve to anchor both a head restraining device and the electrode assembly to the skull. The Kopf rabbit alignment tool (#1244) facilitates alignment of the rabbit skull with lambda 1.5 mm below bregma. After the head is aligned, the electrode is stereotaxically positioned and lowered into place after cutting the dura mater. Even with the alignment tool, surgery can take more than an hour to complete. This necessitates regular supplements of ketamine and xylazine. The supplements are important since rabbits sometimes unexpectedly recover from ketamine/ xylazine anesthesia. Continuous gas anesthesia techniques are an alternative to obviate this risk.

The electrode can be lowered to the exact coordinate, or to an adjusted coordinate determined under physiological guidance. The later technique is preferable. We typically implant electrodes at the dorsal surface of the hippocampus. Once the depth is selected, the hole and guide tube are covered with sterile petroleum jelly before cementing the standoffs to the skull and screws with dental acrylic.

The head restraining device is implanted on top of

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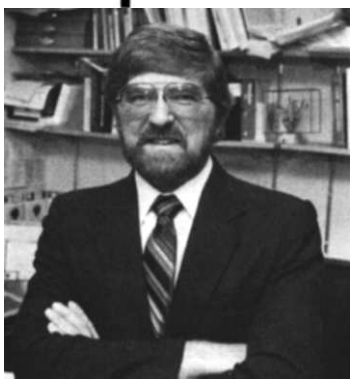

Editor's Column

It is a beautiful afternoon here in Kansas City, City of Fountains. I imagine that most of you readers of the Carrier did not know that Kansas City has more fountains than any city in the world. While there is not one on every corner, there are a lot of them. One big one in particular stands out. It is off the freeway I drive on the way to and from my office every day. On days that the Kansas City Chiefs football team plays, they put red coloring in it, and it sprays red. In the winter, it is kept on, and forms a huge inverted ice bowl, but still some water spray keeps going. I hope the Chiefs keep doing well, especially since there is no baseball now.

Fall means the Society for Neuroscience Meetings. As usual, David Kopf Instruments will have their display there, and we hope to see you during the meeting. Please stop by and chat.

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the skull in front of the electrode assembly. It consists of four nylon bolts (6-32 x 3/4") which project up from the skull. These bolts were previously cemented together while resting in a pool of dental acrylic within the plastic top of a 35 mm film container which served as a mold. These headbolts can be mass produced and sterilized prior to surgery.

RECORDING

We are currently recording the activity of hippocampal neurons during an eyeblink conditioning task (Weiss et al., 1993, 1994). The rabbits are restrained in a Plexiglas box which allows their head to protrude from a stock around their neck. The head bolt is fastened to a fixed plate, and a shielded cable is connected to the electrode assembly.

For those who do not have amplifiers for all of the available wires, the recording cable can be connected to a switch box which allows the output to be selected from any of the available inputs. We use a box with a series of thumb wheel switches to quickly select different wires as inputs without touching the assembly. The same switch box can also be used to change the reference electrode, or to apply current for producing marking lesions.

The electrode cable, or the output of the switch box, is connected to the first stage of an amplifier which contains a field effect transistor (FET) to isolate the signal. These preamps are inside the experimental chamber which is electrically and acoustically shielded. The output of this first stage is then fed into a high impedance AC amplifier for the addition of gain and filtering. The output of the amplifier is fed into an oscilloscope, an audio amplifier, a tape recorder, and a waveform identifier. Other inputs to the tape recorder and waveform identifier include timing pulses for the stimuli that are presented to the rabbits, and a signal which monitors the behavior of the rabbit. The output of an infrared reflective sensor is used to monitor movements of the nictitating membrane (Thompson et al. in press).

The electrode assembly can be lowered further into the brain by turning the screws which couple the connector to the standoff. A #1-72 screw will travel approximately 350 (jm with one full rotation. The assembly is typically advanced by turning each screw 1/12 of a turn in succession. This technique minimizes the cork screw rotation of the electrode assembly that can occur with more rapid advances of a single screw.

SPIKE SORTING

We are currently using the Discovery™ software and hardware interface provided by Datawave Technologies (Longmont, CO, formerly Brainwave) for collecting and separating multiunit activity from each wire into its constituent single unit activity. This software can collect up to 16 channels of data, and then separate the waveforms from each channel by parti-

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tioning parameters of the waveform into unique clusters. The user can observe clustering from up to eight parameters at one time. This information is presented in scattergrams whose points represent the value of the parameters. Delimiting the boundaries of a cluster of points on any one graph simultaneously shows their position on all other graphs. Common parameters such as spike height and width, as well as template matching and principal component matching are available. In theory, the activity from each unique cluster represents the activity from a single neuron. After the spikes are sorted by this "cluster analysis" the waveforms can be displayed for verification of the sorting procedure, and files can be saved which contain the cluster boundaries and the timing of the identified spikes (see Figure 1, pg. 4).

This spike sorting procedure is time consuming, especially as the signal to noise ratio decreases. We are investigating the possibility of using other spike sorting software (e.g. Spectrum Scientific, Dallas, TX) which allows sorting by placing boundaries directly on the waveshape with a "point and click" software procedure, rather than around the values of the wave parameters. Unfortunately, neither of these software applications are currently adequate to fully integrate spike activity with slow wave activity on a continuously recorded data channel. We use such a channel to record the position of the nictitating membrane, others may also require a continuous acquisition mode, e.g. to relate motor neuron firing to force output, or to muscle EMG recordings.

DATA ANALYSIS

The data are analyzed in multiple stages. The first stage is done by the computer that runs the behavioral experiment (Akase et al., in press). This analysis indicates the amplitude and latencies for each response of the nictitating membrane (NM). Trials in which there was a conditioned response, i.e., extension of the NM after the tone but before airpuff onset, are indicated. An average NM response for the session, time locked to tone onset, is then generated. The second stage of analysis involves the spike sorting of the unit data, and then the generation of rasters and histograms for each neuron time locked to tone onset. An average waveform (±1 SD) for each neuron is also generated (see Figure 2, pg. 5). Lastly, routines are being developed to export the sorted file into other statistical programs. Sorted files can be transferred and converted from a PC computer to a binary file on a Macintosh computer using MacLink Plus™ software (DataViz, Inc., Trumball, CT). This file can then be used as input to the Igor™ graphic and waveform analysis package (Wavemetrics, Lake Oswego, OR) to combine the continuous and spike data channels.

HISTOLOGY

After recording the activity of selected cells, or at the bottom of selected tracks, marking lesions are produced by passing current through the recording wire (approximately +50 uA, 10 s). The nichrome wires of the Kubie electrode leave a lesion and gliosis as a result of passing current. The stainless steel wires from the NB Labs electrode result in the deposition of iron ions which turn blue when reacted with potassium ferrocyanide. This is commonly known as the "Prussian Blue" reaction because of the shade of blue that is produced. This latter technique is easier to use for locating recording sites. The reaction is started by perfusion (under deep anesthesia) after a 0.9% saline rinse. A 50/50 solution of 10% potassium ferrocyanide and 10% formalin is perfused through the aorta and then followed by straight 10% formalin. One liter of each solution is used for a 2-3 Kg rabbit. After removing the brain it is placed in a solution of 10% formalin/ 30% sucrose to prevent the formation of ice crystals when the tissue is frozen for cutting. The advantage of carrying out the Prussian blue reaction during the perfusion is that the relevant sections of the brain are quite obvious during cutting on a microtome or cryostat. This allows rapid detection of the recording site and mounting/saving of the fewest sections. The reaction can also be carried out on mounted sections in a staining dish between the steps for cresyl violet staining and dehydrating the slides for clearing and cover slipping.

Prior to cutting the brain it is embedded in a mixture of albumin (30 g/ 175 ml distilled water) and gelatin (3 g, 225 bloom 125 ml warm distilled water). The albumin needs to be mixed slowly, and the gelatin is added after it cools. The mixture is then slowly poured over the brain in a heavy paper mold with the brain correctly positioned. The mold is then exposed to formaldehyde fumes for several hours by placing it on a platform in a tightly sealed glass staining dish with undiluted formaldehyde, i.e., 37%. After the surface of the albumin/gelatin is hardened the mold is immersed in 10% formalin/ 30% sucrose until the brain can be cut.
Embedding the tissue has several advantages. Foremost is that the tissue is easily floated onto a slide in a single sheet. This is especially important at levels of the brain where several pieces may become entangled, e.g. forebrain and cerebellum. The corners of the albumin/gelatin block can also be easily cut, without damaging the brain, to indicate the orientation of the block, e.g. left vs. right. The embedding material is also useful in some experiments combining recording and lesion techniques to indicate recording sites and the extent of lesions relative to intact brain tissue.

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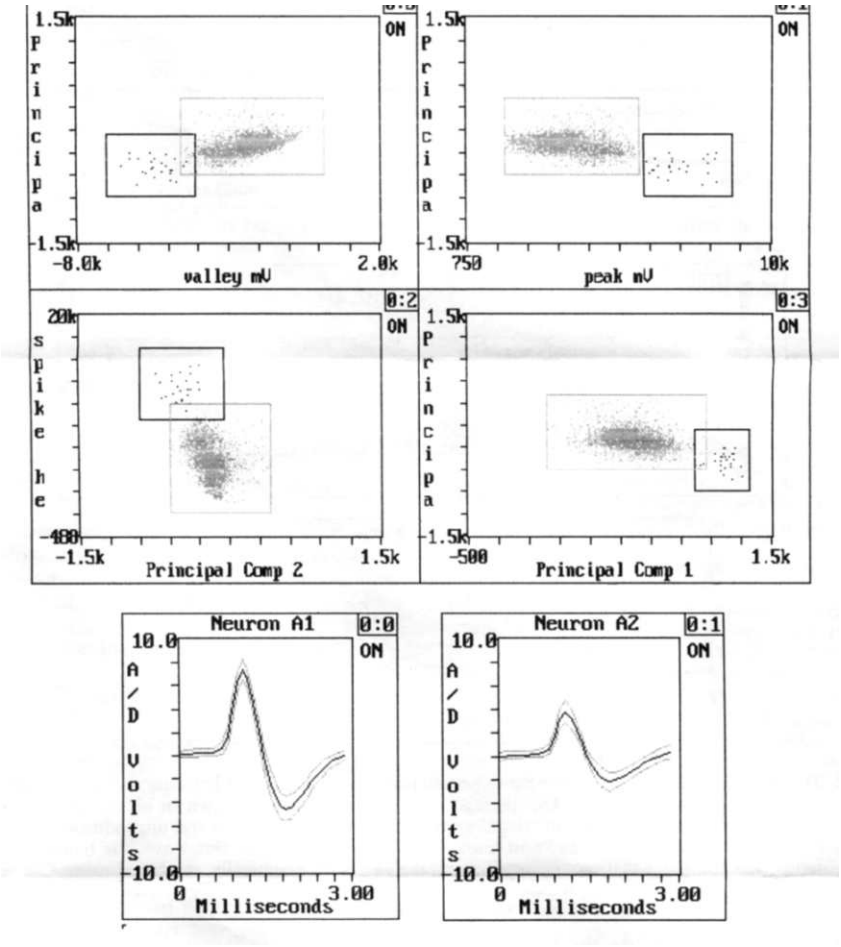

Figure 1. This figure demonstrates the use of "cluster analysis" to separate two neurons recorded from the same electrode. The top four panels show the clustering of neurons 1 (black) and 2 (gray) about 5 different parameters (undiscriminated activity has been omitted for clarity). The bottom two panels show the average (±1SD) waveform for neurons 1 and 2.

COMMENT

Multichannel electrodes provide a means to simultaneously examine the responses and interactions of a population of neurons. Adequate electrodes and recording equipment are commercially available to facilitate this task. Routine histological procedures are available to reliably identify recording sites. Neural recordings may be correlated with slow wave, behavioral, or EMG recordings. The weakest link in currently available commercial software systems is a fast, low cost, standardized routine for correlating large volumes of neural and behavioral data. We are using a suite of high level software routines (macros) in the Macintosh based Igor software program to address this deficiency. These tools are important for future neuroscience research. Currently, major advances are being made in understanding neural mechanisms at the cellular and subcellular level. However, our understanding of brain function at the system level has not advanced as quickly. With more modern approaches, such as addressed here, we are confident that this deficiency can be remedied.

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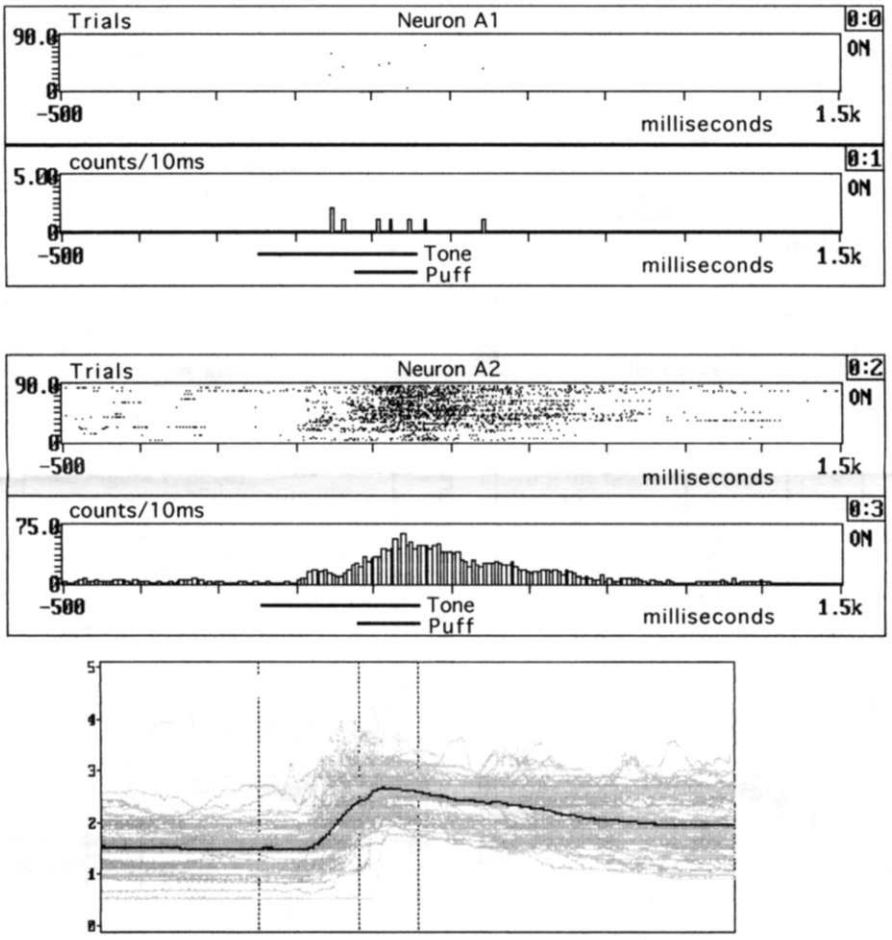

Figure 2. This figure shows the activity of the same two neurons relative to the behavioral responses during a delay conditioning session with 90 trials. The average behavioral response is shown in black; individual trial responses are shown in gray. Note that both single units "model" the conditioned and unconditioned extension of the nictitating membrane. The rasters and unit histograms were generated by Datawave, the behavioral average was generated by the computer that ran the experiment; the two were graphically combined using Canvas™ on a Macintosh computer. An advantage of the Discovery software is that data can be collected for an entire session. This permits the analysis of activity well into the intertrial period.

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