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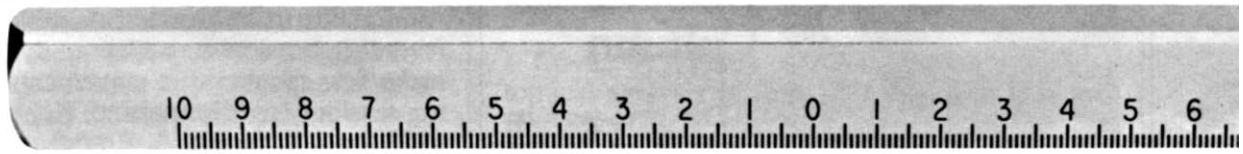

Techniques Used for Neural Recording From Awake, Behaving or Anesthetized Animals Part 2

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Introduction

Over the past several years, the design of stereotaxic 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, emotions, and cognition. For example, in the area of learning and memory, intracellular techniques have been used to study behavioral plasticity in invertebrates like *Apfysia* (e.g., Kandel & Schwartz, 1982) and *Hermissenda* (Parley & Alkon, 1982) while extracellular recording methods have provided some insight into the neuronal circuitry, activity, and plasticity associated with a variety of mammalian preparations such as eyelid conditioning (e.g., Thompson, 1986), VOR training (e.g., Lis-berger, 1988), 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 Gorme-zano, 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 microstimulation, lesions, pharmacology and anatomical methods. The first part of this article appeared in the previous issue of the *Carrier* (October 1991) and may be obtained from David Kopf Instruments. In that section of the article, methods for electrode manufacture, stereotaxic surgery, and electrode and cannula implantation were presented. In this section, neural activity and eye muscle recording techniques, histological, and data capture and analysis methods are given.

Neural Recording from the Awake, Behaving Rabbit

The majority of research conducted in our laboratory involves multiple-unit or single unit recording from awake, behaving animals over a number of sessions. Specifically, we have monitored brain activity during classical eyelid conditioning in rabbits and during signalled-bar pressing in rats. A description of the behavioral/neural

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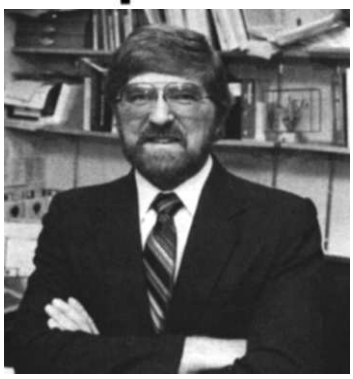

Editor's Column

The new year is upon us. It hardly seems possible that 1991 is over as I write. Maybe as I get a bit older, time goes faster. Perhaps the perception of time with aging would make a good research area. At any rate, as we look back at 1991, we can see many changes in the world which will affect us for the foreseeable future.

The biggest of these changes would surely be the breakup and reformation going on in the old Soviet Union. This immense upheaval has the potential for great good or great harm, depending on which way the new government goes. Part of that will be decided, I am sure, by the response of the West to the plight this winter of the Soviet people. If they get the aid they need to make it through this time, they will embrace a market society. Otherwise, they are likely to embrace, through civil unrest, another totalitarian regime which at least promises bread and milk for the table. We hope the West responds appropriately.

Other things we can see which affect us as scientists include the continued push of the animal rights groups to make research harder and more costly. There are some hopeful signs in this ares, such as laws protecting research facilities and making it a crime to destroy labs and steal animals, but the publicity using false and misleading statements continues, with prime time TV and newspaper ads which reach a lot of people. We must continue and expand our efforts to educate the public about the benefits of research and science for all of the world.

So, as the old year recedes into memory and a new one dawns, full of hope and possibilities, but beset by problems and difficulties, let us all resolve to treat each other, our fellow world inhabitants and our Mother Earth with more respect.

HAPPY NEW YEAR!!!

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recording systems we use is presented here.

Rabbits make wonderful subjects for awake recording because they adapt to restraint well and make few spontaneous movements during training sessions (see Gormezano, Kehoe & Marshall, 1983, and Patterson & Romano, 1987, for reviews). During classical eyelid conditioning, rabbits are restrained in Plexiglas boxes that have been built at Indiana University. These boxes surround the rabbit on all sides and provide a head stock and ear-clamp design that minimizes head movement. We have found that the mild restraint provided by this type of restraint box is sufficient for all types of recording. Even though the head is not bolted or secured to devices surrounding the rabbit, using the Narishige Model MO-99 microdrive system, we have managed to isolate and record single-unit activity from brain regions like the cerebellum, hippocampus and caudate nucleus over several blocks of training. Some units have been held as long as 1 hr. Conditioning sessions are conducted in sound-attenuating chambers that have been outfitted with exhaust fans (that supply background, white noise), speakers for delivering auditory stimuli, depowered lights that can be used as a stimulus, and an air hose that originates from a solenoid-controlled valve located in an adjoining room. A computer system programmed in Forth and machine code controls the delivery of stimuli (via standard interfacing strategies such as optoisolators and relays) as well as directs the collection of behavioral and neural data (Lavond & Steinmetz, 1989).

In the past we used minitorque potentiometers or photodiode devices to record movement of the nictitating membrane or outer eyelids. More recently, however, we have monitored eyelid movement by recording EMG activity from the musculature surrounding the eye. We feel this method has two advantages. First, this system eliminates the need for placing large assemblies on the top of the head for mounting recording apparatus (we now only mount a block or screw to attach a nozzle for delivering the air puff). Second, EMG recordings are very sensitive and free of mechanical artifacts that are sometimes involved in transducing eyelid movements. The EMG signal is recorded from the stainless steel wires that were implanted during surgery (see above). The signal is picked up through gold, female Amphenol pins that match the male pins crimped to the stainless steel wires during surgery. The EMG signal is input to an A-M System Model 1700 amplifier located outside the cham-

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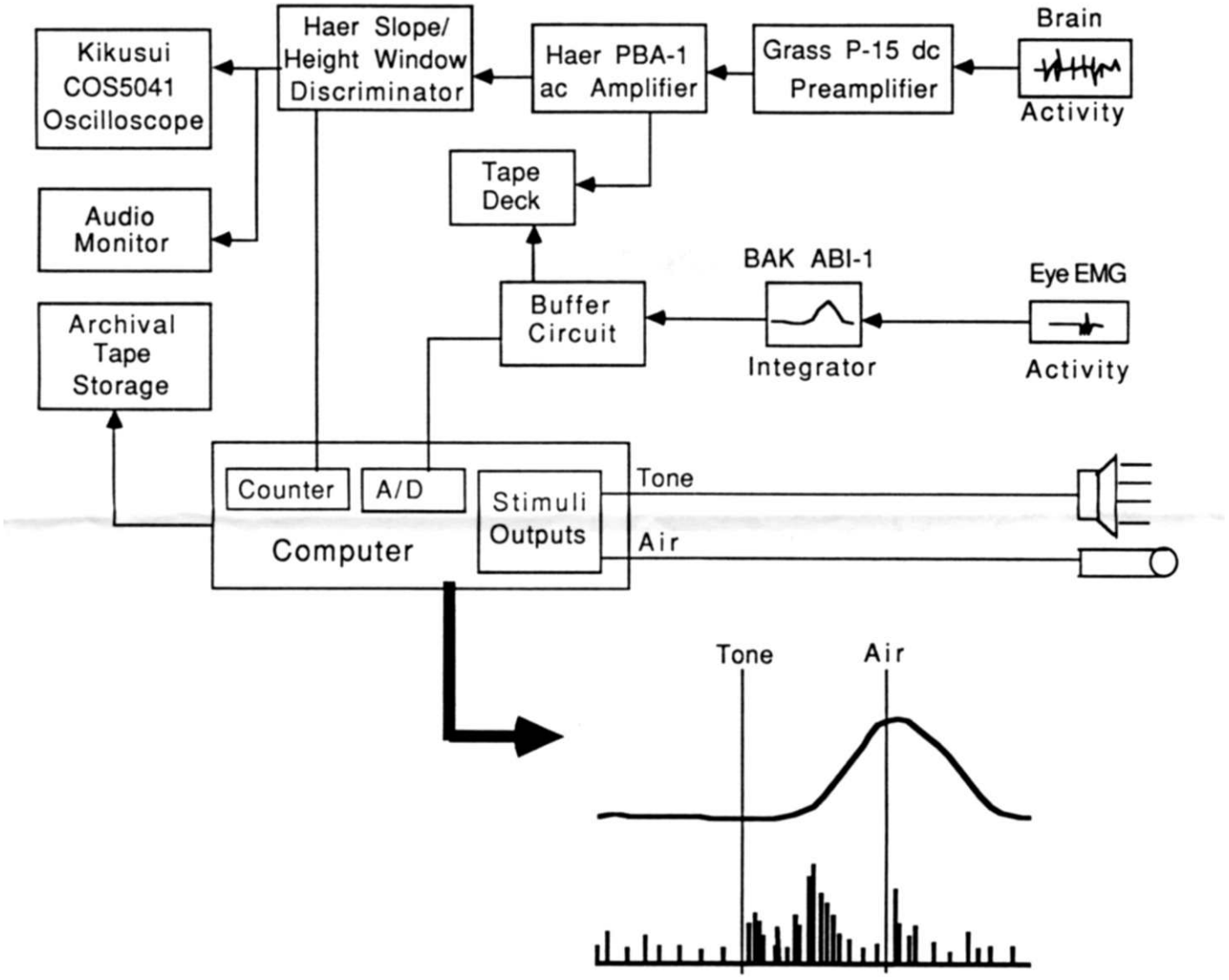

Figure 1. Schematic view of the apparatus used to provide a tone and air puff stimulus to the rabbit ject, and to record the EMG of the eye musculature and the brain activity present during the conditk session. A typical single trial output is shown at the bottom, giving the brain activity and the integrated EMG response

ber which amplifies (100X) and band pass filters (100-5000Hz) the signal. Output from the amplifier is then routed to a BAK ABT-1 AC Bridge Integrator that converts the filtered EMG signal to a dc signal that is eventually scaled between 0 and 5 V by a buffering circuit that allows the experimenter to designate a specific baseline for the integrated EMG signal. The dc signal is then input to a A/D in the computer which monitors the behavioral response between and during training trials (see Figure 1 for a schematic of the stimulus and recording equipment).

Multiple- or single-unit activity is recorded from electrodes that were either implanted during surgery or lowered during the session (see above). We use a two-stage amplification system for unit recording. The brain signal is first sent to a Grass de-powered P15 amplifier that is located within the chamber. This amplifier provides a 10-100X gain and filters the signal between 300 and 10,000Hz. Connections from the preparation to the P15 are made via cables with gold, female Amphenol pins soldered to the ends. Output from the P15 is then routed to a Haer PBA-1 ac amplifier (gain=10-100X; filters=500-5000 Hz) which in turn routes the signal to a Haer Slope /Height Window Discriminator that allows spikes to be discriminated at levels set by the experimenter. This discriminator allows three comparator levels to be set and also creates two windows for spike discrimination. The multiplexed output of the discriminator is viewed on a Kikusui Model COS5041 analog oscilloscope. The TTL pulses that represent discriminated action potentials are routed to a counter/timer chip in the computer

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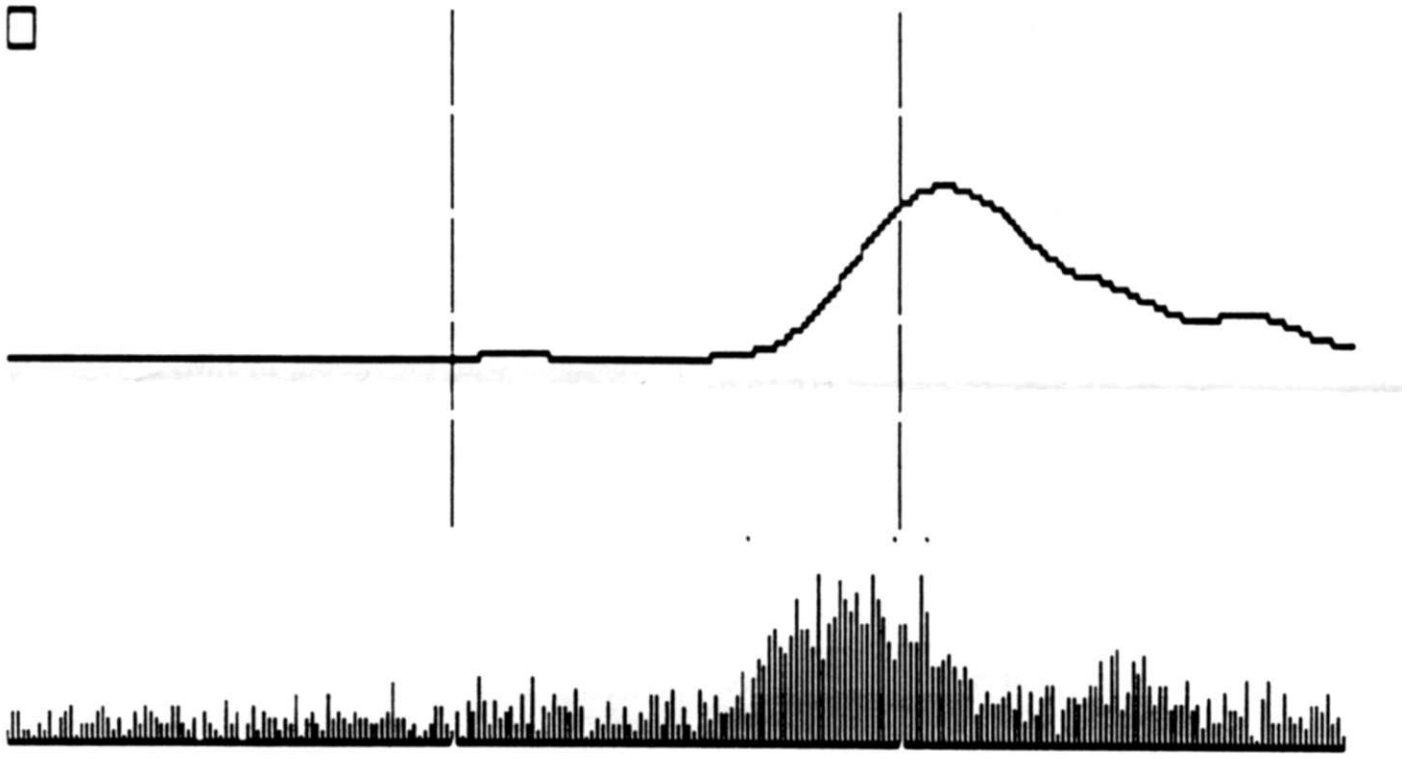

Figure 2. The behavioral and neural output from a well-conditioned rabbit. The behavioral output is shown at the top averaged over several trials. Note the lack of noise in the behavioral record. Averaged multiple unit activity from the hippocampus is show on the bottom, with each line representing counts per 3 millseconds. Note that the multiple unit activity begins to rise well before the behavioral response.

which compiles spike counts within predefined time intervals during training trials. We have also used this sytem to generate peristimulus time histograms of unit activity evoked by auditory or electrical brain stimulation in the awake rabbit.

Whenever possible we record raw unit activity on one channel of a TEAC Model V-250 stereo cassette tape deck. A timing pulse issued by the computer at the beginning of each training trial is recorded on the second channel. During each session on a trial-by-trial basis, the computer stores A/D data representing the behavioral response as well as histograms of discriminated neural activity. These data are displayed graphically during training sessions and subsequently blocked and analyzed after the session is complete (using summary program written in Forth as well as data analysis routines prepared for standard spread sheet programs). All data are archived on a Tecmar QT60e tape system for additional analyses.

Histology Techniques

After data collection has been completed for each animal, it is necessary to use histological methods to verify the location of recording electrodes, stimulating electrodes, or cannulae, or to examine the extent of a lesion placed in the brain. Before euthanizing the animal, we measure the impedance of each electrode with the BAK electrode impedance tester to insure that the impedance properties of the electrodes have not changed significantly during the course of the experiment. For recording and stimulating electrodes and cannulae, we next mark their position by passing 100 |iA dc for 10 seconds with a Grass DC constant current lesion maker. We then

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overdose each animal with an iv injection of pentobarbital and perfuse the animal via the ascending aorta with about 1 liter of 0.9% saline followed by 1 liter of 10% formalin. The brain is then removed from the skull and placed in a jar of 10% formalin/30% sucrose solution until it sinks to the bottom (about 1 wk). The brains are then quick-embedded in an albumin/gelatin solution that contains glutaraldehyde as a hardening agent. The brains are then blocked and frozen on a microtome stage using dry ice. Next, 50-80 (im slices are taken, transferred to a 20% alcohol solution, then mounted on gelatinized slides. After a 1-2 day drying period the slides are stained with potassium ferrocyanide (for traces of iron left behind by the lesions) and cresyl violet (for Nissl substance). A variety of protocols are available for staining. However, it is our experience that care must be taken in performing three steps of the staining process. First, after exposing the slides to potassium ferrocyanide, the slices must be adequately neutralized in a sodium acetate solution. Second, the amount of time the slides are kept in the cresyl violet is dependent on the age of the cresyl violet solution and must be carefully monitored. Third, because the amount of time the slides spend in the differentiator (typically a few drops of glacial acetic acid in alcohol) varies, the slides must be carefully monitored for proper contrast. After staining, the slides are cover-slipped using Permount solution and set aside to dry. After about 1 week, the slides can be viewed under a microscope to determine electrode position or lesion extent.

Concluding Remarks

We have presented several experimental techniques which we have used mainly for recording neural activity from awake, behaving animals. We have given mainly the neural techniques-see Gormezano, *et.al.,* (1983), and Patterson and Romano, (1986) for a review of the behavioral aspects. Even though several methods are outlined above, this is by no means an exhaustive review of methods used by researchers in this general field of study. We recommend that the reader examine journals such as *Behavior Research Methods, Instruments, & Computers, Physiology & Behavior, and Journal of Neuroscience Methods* for current advances in experimental techniques. Additional information concerning methods described above can be obtained by writing to J. E. Steinmetz, Department of Psychology, Program in Neural Science, Indiana University, Bloom-ington, IN, 47405.

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