PROGRESS IN PSYCHOBIOLOGY AND PHYSIOLOGICAL PSYCHOLOGY. VOL. 15

Brain Neuronal Unit Discharge in Freely Moving Animals: Methods and Application in the Study of Sleep Mechanisms

Dennis McGinty* and Jerome M. Siegel†
Sepulveda Veterans Administration Medical Center
Sepulveda, California 91343
and
Departments of Psychology and Psychiatry†*
University of California, Los Angeles
Los Angeles, California 90024

I. Introduction

In the evolution of hypotheses concerning brain mechanisms of behavior, few techniques have had more influence than the study of neuronal discharge in awake behaving animals. The basic principles behind this approach are simple. Any set of neurons hypothesized to be involved in a particular function can be expected to exhibit altered discharge in temporal relationship to the occurrence of the function. In addition, the details of the temporal patterns of discharge correlated with the function could provide specific information about the kind of complex coding in the neural circuitry underlying the associated brain mechanisms. Well-known examples include movement-related cells in the precentral cortex and stimulus "edge" detectors in the visual cortex.

Outside of delineated sensory or motor pathways, many brain areas appear to have multiple functions, possibly reflecting the various specializations of closely adjacent or overlapping subsets of neurons or the convergence or integration of multiple functions by individual neurons. The functions of any particular neuron encountered either electrophysiologically or histologically may be unknown. In these cases, unit-recording techniques may allow us to identify such functions. For example, we may ask whether certain hypothalamic neurons show changes related to satiety, hunger, or food ingestion, or physiological stimuli related to feeding (e.g., blood glucose), rather than to any of several other hypothalamic functions.

Even certain concepts with a primarily neurochemical basis may be studied by examining neuronal unit discharge. This approach is based on the concepts that the release of neurotransmitters is initiated by nerve impulse flow and that the neuronal discharge rate is, therefore, one measure of the rate of transmitter release. This method can be used when chemically defined neurons (e.g., serotoninergic neurons) are clustered such that they may be localized using conventional histology, or they have unique identifiable electrophysiological properties, inputs, or projections. The role of monoaminergic mechanisms in sleep was studied by recording the discharge patterns of identified monoamine-releasing neurons. Examples of this type of experiment are summarized below (Section IV,D,G).

Clearly, a variety of methods play essential roles in the analysis of brain functions, and each has both uses and limitations. Neuronal recording, in comparison to lesion, stimulation, biochemical correlative, pharmacological, or imaging techniques, can provide more specific information concerning anatomical localization and temporal features of control mechanisms. Motor control systems may provide the clearest example. A lesion study of a particular part of the brain may reveal a motor deficit, but a unit-recording study could reveal discharge related to a specific element of movement, such as initiation or termination of movement, movement velocity or force, to learned or unlearned aspects, or even to certain motor fiber types. Neurons with a specific relationship to movement may be localized in a specific subregion or layer of a structure. Thus, neuronal unit recording provides unique information about both detailed temporal and spatial coding and relationships to subunits or elements of a behavior. We may refer to this information as the "behavioral functional neuroanatomy of a brain site."

On the other hand, neuronal unit-recording methods are essentially correlative in nature. It is very difficult to know whether activity in a particular neuronal type is generating a process, or only responding to some input which is the true generator. When events or processes have abrupt onsets with latencies of responses in the millisecond range, as, for example, in the case of a startle response, it may be possible to make interpretations that include causal relationships. Such interpretations may be useful in the context of specific anatomical information concerning probable neuronal connections. However, in the case of behavioral events such as sleep-waking state changes, which occur over the course of several seconds or more, latency information is far less definitive relative to questions of causality.

Thus, lesion or stimulation methods have more often provided the bases for generating hypotheses about the role of a particular brain site in a function. However, neuronal activity studies may be used to verify and refine a hypothesis, to suggest particular ways in which a mechanism is regulated. Still more reductionistic methods, including analysis of receptors and membrane conductance mechanisms, neurochemical synthesis, release, binding, and inactivation, and

details of neuronal structure, will eventually be brought to bear on any particular problem. However, it should be clear that understanding of the functions and mechanisms of any brain structure will also depend on knowing exactly when neuronal subtypes are active. We believe that this behavioral functional neuroanatomy in any site is as crucial to understanding of the site as the chemical anatomy or the identification of transmitters or projection patterns. It would seem that only with a combination of these approaches can the mechanistic basis of behavior be determined.

In this article, we examine the status of investigation of sleep mechanisms and assess the uses (and abuses) of chronic unit-recording studies for the larger goal of unraveling the nature of sleep mechanisms. From the start, we focus on attempts to understand behavior. In the case of sleep, we hope to determine what mechanisms explain processes such as the change in behavior defined by the onset and termination of sleep, the stages within sleep, including rapid eye movement sleep (REMS), the temporal features of sleep architecture, the regulation of the amounts of sleep, and the unique features of physiological processes within sleep. While these goals have not been achieved, the analysis of sleep mechanisms has developed to the point where specific mechanistic hypotheses are under study.

The study of brain mechanisms of sleep has evolved along a course not unlike that of other types of behavior. Initial beliefs that mechanisms might be associated with restricted cell groups such as norepinephrine- or serotonin-containing neurons have given way to more complex concepts. At the same time, there has been an astonishing growth in the knowledge of more basic neurophysiological mechanisms. We are now aware of dozens of neurotransmitters and neuromodulators, receptors with multiple components, complex pre- and postsynap-tie modulatory events, and a variety of ionic processes regulating cellular excitability. Each cell seems to be capable of intricate adaptations. Each region of the brain contains numerous cell types. In the current context, it is not surprising that different methods used in the analysis of structure-function relationships have sometimes yielded conflicting results. Of particular interest to students of neuroscience may be a comparison of the conclusions obtained by different methodologies.

II. A Chronic Neuronal Unit-Recording Method

To apply neuronal recording techniques to the problems of behavioral functions, it was first necessary to study the unanesthetized animal. This approach was pioneered by Hubel (1960), Jasper *et al.* (1960), and Evarts (1968) in head-restrained animals. As researchers interested in certain slowly changing behaviors and sleep and waking states, we required a technique that would permit long-

term stability of unit recording. Further, we were interested in brain areas thought to be involved in a wide variety of behavioral functions, and we anticipated that the use of head restraint would severely limit the kinds of behavior we could study. These were the technical issues we faced in 1970. We developed a simple technique that permitted chronic unit recording in unrestrained animals for hours, sometimes days. The method is based on the use of fine-wire recordings of unit discharge. This article summarizes the technical aspects as well as the applications of the method, using examples from our work in sleep neurophysiology. Among the most crucial findings have been the analysis of movement-related discharge in medial reticular formation neurons (Siegel, 1979a), the long-term cyclic REMS-off behavior of dorsal raphe serotonin-containing neurons (McGinty *et al.,* 1973; McGinty and Harper, 1976), and the sleep-selective activity of neurons within the basal forebrain (Szymusiak and McGinty, 1986b). A review summarizing many of the findings of this approach in a wider range of behaviors is also available (McGinty and Szymusiak, 1988a).

Prior to our work, Strumwasser (1958) and Olds *et al.* (1972) had demonstrated the usefulness of chronically implanted, fixed-position, "floating" fine-wire electrodes in providing stable unit or multiunit recordings in unrestrained rodents. This method had also been used successfully in cats (Naka and Kido, 1967;O'KeefeandBouma, 1969; Harper, 1971; Burns *et al.,* 1974) and humans (Marg and Adams, 1967). However, fixed electrodes are obviously limited, since recordings are restricted to neurons close to final resting site of the electrode after implantation. One of us (D.M.) developed the simple, skull-mounted, light-weight microdrive that permitted the exploration of a track through a site, like that used with conventional rigid microelectrodes. This improvement made it possible to record from many more neurons in a single animal and to adjust electrode position to optimize isolation of action potentials from single neurons (Harper and McGinty, 1973).

Neurophysiologists who had struggled with the construction and use of very high-impedance microelectrodes were initially skeptical that electrodes with exposed tips several times larger than those of conventional microelectrodes could possibly isolate single units. However, results provided by fine-wire techniques have been completely consistent with those obtained using conventional microelectrodes; the method has stood the test of time. The long-term stability of unit recording with microwires has permitted certain types of experiments that were very difficult or impossible with conventional microelectrode techniques. This feature has led to a number of discoveries about the coding of behavior. While more complex variations of the microwire method have now been described (e.g., McNaughton *et al.,* 1983), and these may be useful in some experimental problems, our particular method still offers an advantage in simplicity. A detailed discussion of the biophysical and other technical issues involved in chronic unit recording can be found in the monograph by Lemon (1984).

The features of our method for recording neuronal unit discharge in unrestrained animals are shown in Fig. 1. The electrodes consist of fine insulated wires, commonly called microwires, which are threaded into the brain through the barrels of stainless steel cannulae that are part of a microdrive. The micro-drive is described in detail below. Unlike conventional electrodes, which are relatively thick except at the sharpened tip, microwires have the same small diameter throughout their length. This makes it possible to place several adjacent electrodes in a site, driven from one microdrive, without producing extensive damage to adjacent tissue. Since a cluster of microwires is used and calls can be studied from each wire, a single pass with the microwire bundle is equivalent to several passes with a conventional single microelectrode. In addition, it is often possible to record from two or more neurons in close proximity, permitting the analysis of interactions of adjacent cells (see below). The animal can be prepared with a variety of additional recording electrodes or other devices, such as for recording electroencephalographic (EEG) patterns, eye movements, and muscle activity and for brain stimulation (see Fig. 1 and further discussion below).

Additional advantages are derived from the fact that the microwire electrodes have a lower impedance compared to conventional microelectrodes. This means that recordings are less influenced by "antenna-derived" noise and by electrical artifacts generated by cable movements. Thus, study of discharge during all types of movement of animals is possible. Conventional microelectrodes can be used in unanesthetized animals with the head rigidly restrained, but this creates certain complications. The animal must be adapted to the restraint, but even with adaptation the procedure could remain stressful. Further, head restraint prevents head movements and locomotion, two basic classes of movement.

Stability of recording is usually the greatest difficulty encountered when using conventional microelectrodes. The stability of microwire unit recording is among its greatest advantages. Instability is thought to be caused by slight movements of brain tissue relative to the electrode tip, and may result from "pulsations" in blood flow or movements related to respiration. These effects are amplified if the skull cavity is open, as it is with conventional microelectrodes. However, with the microwire technique, electrodes are implanted chronically and the skull is sealed, minimizing such movements.

Two variations on the method for placing the microwires within a guide cannula are in use in our laboratories. The original method utilized a bundle of microwires prepared before surgery. The advantage of this method is that each wire can be soldered to an electrical connector prior to surgery, eliminating a difficult step during the surgical procedure. The bundle is lowered through the cannula as a unit. Histological inspection of electrode tracks indicates that wires of a bundle often remain in a compact cluster within the brain. It is not unusual to record the neuronal discharge of the same cell from two, presumably adjacent, wires. When 32-/um or finer wires are used in the bundle, it may be useful to add

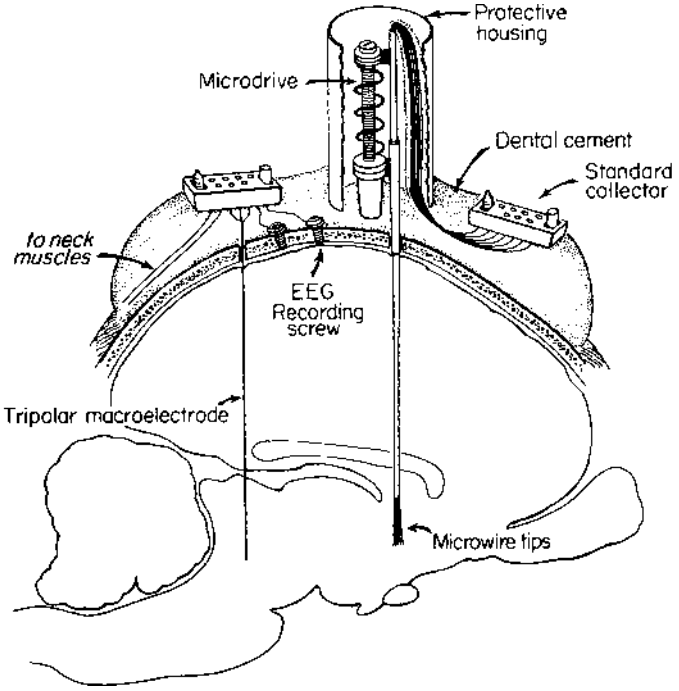


FIG. 1. Schematic view of the cat brain and overlying skull showing how the microwire unit-recording system is mounted. The microdrive is stereotaxically positioned in a selected brain site and fixed to the skull with dental cement. Additional recordings from cortical surface electroencephalographic and electromyographic electrodes or from depth electrodes may be made through separate electrical connectors.

stiffness to the bundle by including nonrecording 65-/um wires that end about 5 mm from the bundle tip.

A second method consists of lowering three to seven microwires individually through the guide cannula during surgery, which was done to achieve two goals. First, we found that bundles did not readily penetrate ependymal tissues in the floor of the fourth ventricle, because of the combined cross-sectional area of several wires. Individual wires could penetrate readily. When individual wires were cut on an angle, to provide a beveled tip, they penetrated tissue even more easily. However, individual 19- to 32-/um wires do not have sufficient stiffness to be threaded through a cannula into the brain. In order to insert single fine bevel-cut wires individually, it was necessary to attach a strengthening support wire. This was done by gluing 37-, 45-, or 65-/um support wires to the finer recording wires, the latter extending 1-4 mm beyond the end of the support wires (see below). The glued microwire pairs typically have a slight curvature. This

yields a second advantage to this variation. The tips of the microwires tend to follow slightly different paths, providing some dispersion of the tips, and a slightly wider area of neuronal sampling under the cannula. A third use of individual microwires has been suggested (Chang *et al.,* 1988). In some species, during neck movements, the medulla moves longitudinally relative to the overlying cranial bones, to which the microdrive is fixed. Therefore, recordings of medullary unit discharge in unrestrained chronic animals are very unstable. Chang *et al.* proposed that the individual fine wires may "float" in the medulla even during longitudinal movements, permitting improved recording stability.

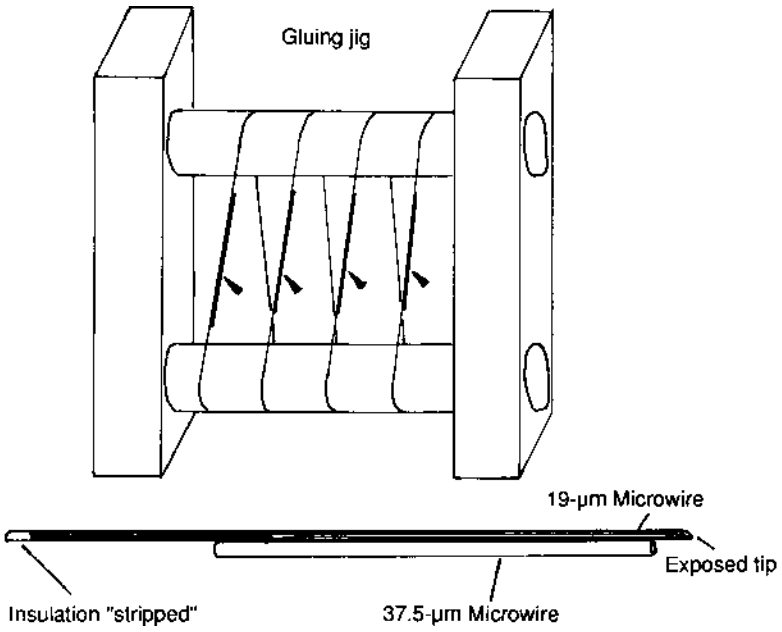
The disadvantage of the individual-wire method is that the fine wires must be soldered to a connector after placement in the microdrive, a critical but tedious and sometimes difficult task that must be added to the surgical procedure.

Formvar-insulated stainless steel microwire can be purchased in any diameter and in a variety of alloys from a commercial source.[1] It is important to order double- or triple-insulated wire that is "stress-relieved" and shipped on a 6-inch spool, to maximize wire straightness. We currently use type 304 stainless steel because it is relatively stiff and contains iron. The iron content permits the deposition of iron with an anodal current and the identification of the electrode tip with the Prussian Blue staining technique (Siegel, 1968). We use wires with diameters of 19, 25, 32.5, and 37.5 /um for electrodes, and 37.5, 45, or 62.5 /um for support wires, depending on the nature of the recording site. In many sites, the larger wires are as effective as smaller wires in achieving good recordings. In some sites, a combination of 32- and 65-/um wires has proved effective.

Wires to be placed in the brain as part of a bundle are prepared by cutting desired lengths (e.g., about 110 mm for the cat). If wires are to be introduced glued to support wires, several "coils" of electrode wire are wrapped on a jig (Fig. 2). The support wires are then glued to the suspended portions of the electrode wire. We use Epoxylite (Epoxylite Corp., S. El Monte, CA) enamel glue, which requires that the entire jig is baked for about 1 hr at 100°C. The glued pairs of wires are then cut free in 110-mm lengths, the support wire ending about 2-4 mm from one end. This end is prepared as the recording tip. The recording tip can be cut either at 90° or at a 45-60° angle to create a pointed electrode. The opposite end must be stripped of insulation (2-3 mm) to facilitate soldering to a head-mounted connector. Insulation is removed with fresh commercial enamel remover (Strip-X, GC Electronics, Rockford, IL) or by burning off insulation with a small flame (Chang *et al.,* 1988). It is also important to "tin" this end, using stainless steel solder and flux prior to surgery. We have recently started to electroplate gold on the recording tip[2] to produce a more

[1]California Fine Wire. 338 South Fourth Street, P.O. Box 446, Grover City, California 93433; (805) 489-6760.

[2]EuteeSol Flux, type 682, Euteetic Corp., 7731 Oakport, Oakland, California 94621; (800) 662-0051.



FIG;. 2. Technique for making individual microwires with support wires. (Top) Several turns of the recording wire are firmly wrapped on a wood-gluing jig. Sections of the selected support wire (about 60 mm long for cats) are dipped in Epoxylite and positioned on the suspended sections of the recording wire. After "baking" for about 60 min at 100°C to harden the epoxylite, sections of wire are cut free (bottom).

chemically inert exposed surface, although we have not done a controlled study to determine whether this is important.

We emphasize that the preparation of the microwires is a crucial aspect of the procedure; microwire electrode failure is not uncommon. The most common types of failure appear to be (1) poor soldering of the very fine stainless steel wires to the connector, (2) insulation failure along the microwire, and (3) poor configuration of the electrode tip. It is important to use stainless steel solder and flux.[3] When using 25-/um or smaller wire, it may be useful to take additional steps to ensure a good solder joint such as soldering tiny pieces of tubing to the wires before surgery. The cutting of the recording tip should be done with very sharp scissors. The properties of microwire insulation and tip configuration may be indicated by the electrode impedance. We have begun pretesting the impedance of microwires after implantation to reduce the failure rate. A commercial

[3]Electrode impedence meter, model BL-2000, Winston Electronics, P.O. Box 638, Millbrae, California 94030; (415) 589-6900.

microwire impedance tester is available.[4] Wires with either too high or too low impedance should be replaced. Insulation failure typically develops over a period of 1-2 weeks. Diana *et al.* (1987) compared the effectiveness of different insulators *in vivo* and concluded that Parylene (Union Carbide) or lacquer were superior to Formvar, but they did not test double- or triple-layer Formvar, which we have found suitable.

The design of the microdrive was determined by the requirement for compactness; we wished to be able to mount several drives on one animal, while maintaining space for electrical connectors to accommodate each microwire plus stimulating and recording macroelectrodes. We also wished to avoid the need for specialized machine tools. We continue to use the original microdrive, built with less than $1 worth of readily available hardware items[4] in less than 30 min (Fig. 3). More refined, machine-made, microdrives can also be used. A small metal block can be drilled with adjacent holes serving as guide tubes and, after threading, providing the positioning of the machine screw. With this approach, the acquisition of the slight skills needed to master the construction procedure can be avoided, but there is little or no improvement in results. However, one advantage of machine-made microdrives is the possibility of creating three-dimensional arrays of cannulae, driven by the same machine screw.

The steps in constructing a microdrive are shown in Fig. 4. Using a grinding disk on a moto-tool, stainless steel tubing sections for both inner and guide cannulae can be cut to appropriate lengths, as determined by the depth of the recording site below the dorsal surface of the skull. Stainless steel tubing sizes (gauges) for the inner and guide cannulae can be 24TW and 21TW or 23TW and 20TW, respectively. Three to five stainless steel nuts are tightened (using fingertips) on a machine screw and soldered together to form the stack (Fig. 4B). After the outer cannulae are positioned in a suitable jig (Fig. 4C), the nut stack is soldered to them. The machine screw used during this soldering operation can then be removed and saved for the next fabrication. A new screw is inserted through a stack of three washers, and the spring is then threaded into the nut stack for several turns, the spring now being compressed against the washers. The inner cannulae are then slid through the outer cannulae into position and soldered to the washers (Fig. 4D). Microdrives can be constructed with one, two, three, or more cannulae. A final step is to cement in place a piece of flexible tubing that serves to maintain a path under the nut stack for the machine screw. Otherwise, this area may inadvertently be filled with dental cement during surgery. Microdrives built for use with rats, cats, and dogs are shown in Fig. 5.

An important advantage of the handmade microdrive is the ability to build a wide variety of configurations of the cannula positions in order to optimize

[4]Small Parts, Inc., 6891 NE Third Avenue, **P.O. Box** 381966, Miami, Florida 33238-1966; (305) 751-0856. Catalog available.

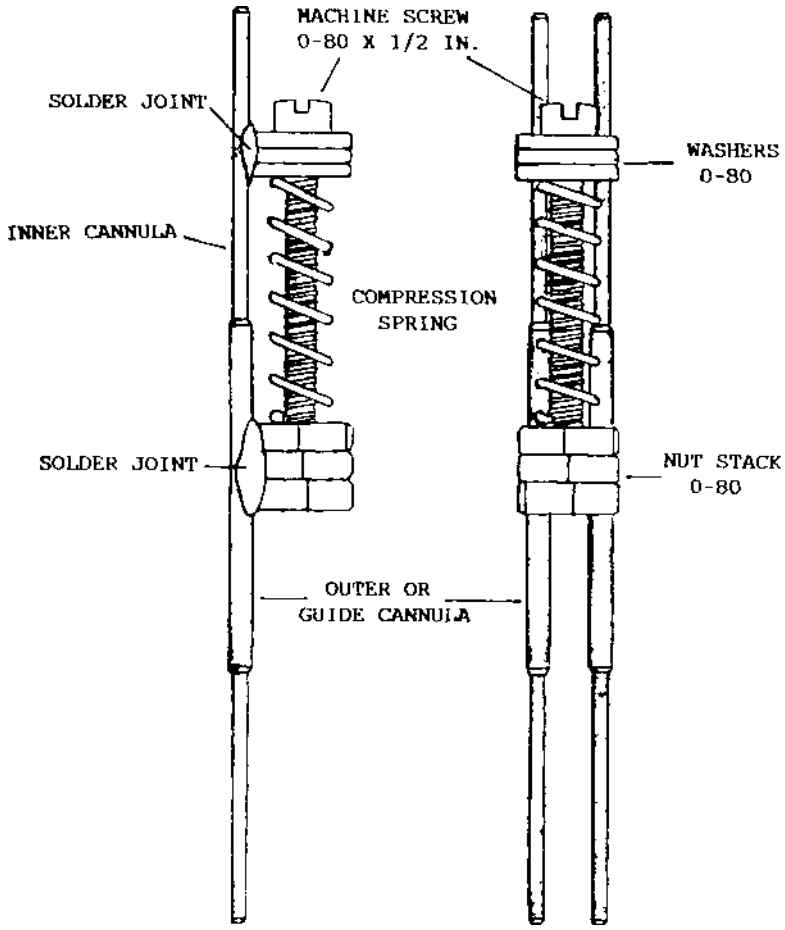


FIG. 3. Details of the mechanieal microdrive. Shown is a two-barrel microdrive with barrels separated by 2 mm. Both the number and the separation of barrels, as well as their length, can be varied according to the needs of the experiment. For additional details, see text and Fig. 4.

recording from neuronal groups with varying spatial dimensions. Additionally, it is possible to build microdrives with recording sites adjacent to sites for microinjection, measurement of temperature, and electrical stimulation or for any other implantable device. The adjacent device can move in parallel with the microwire tips, or not, according to the needs of the experiment. Figures 6 and 7 show two variations for microinjection adjacent to recording sites. Experiments utilizing cholinergic stimulation adjacent to recording sites are described below (Section IV,E). In this case, microinjections were made about 1 mm from recording sites and utilized an

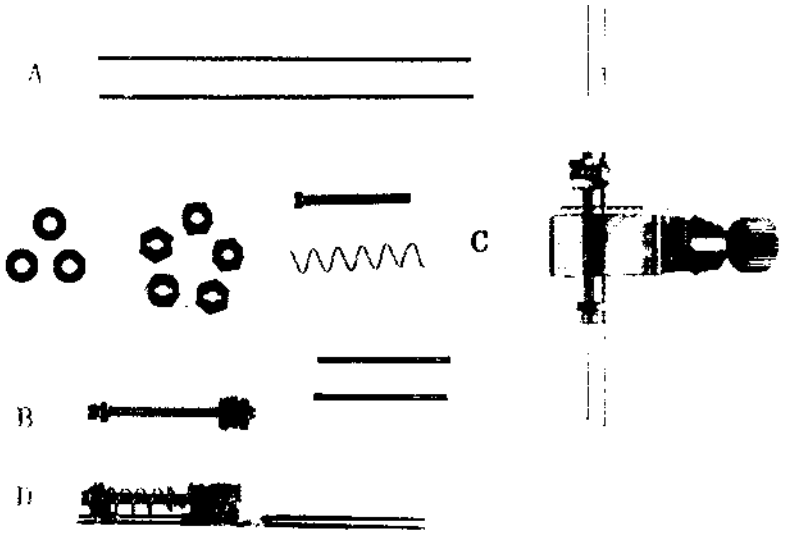


FIG. 4. Construction of a two-barrel mechanical microdrive. (A) The parts required include stainless steel tubing for the inner cannula and the outer or guide cannula (below spring), washers (size 0), nuts and machine screw (size 0–80), and compression spring. (B) Three to five nuts are tightened together on a machine screw and soldered to form the nut stack. (C) A stereotaxic electrode holder is used as a jig to hold the inner cannulae parallel at the desired separation. (D) Final configuration of the microdrive (see text).

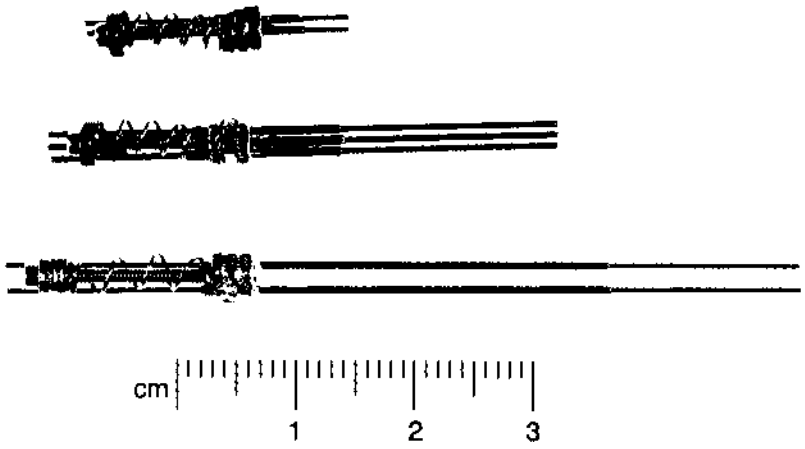
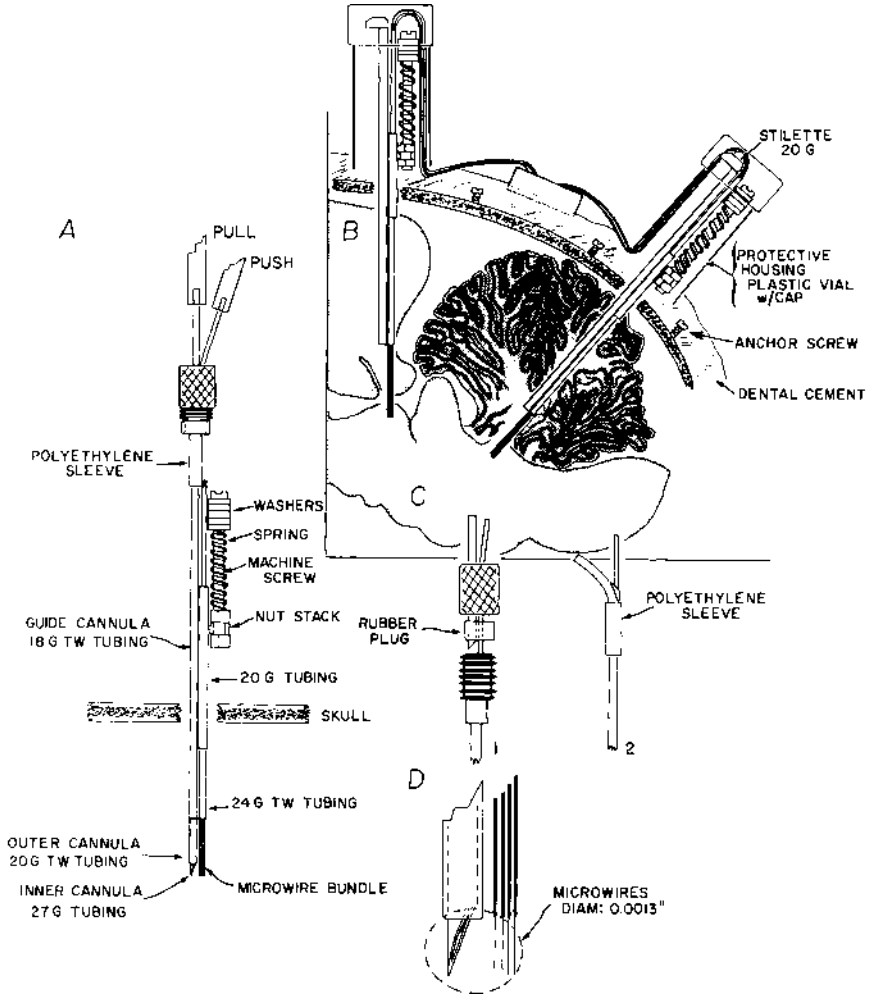


FIG. 5. Microdrives for deep recording sites in the rat, cat, and dog (note scale at bottom). Three different barrel configurations are shown.



FIG,. 6. System for microinjection or chemical perfusion adjacent to microwire unit-recording sites, illustrated for the cat midbrain (B) and pontine (C) sites. (A) Diagram of a push-pull cannula system attached to the microdrive. The push-pull system guide cannula is positioned beside the microdrive so that the tip is about 1 mm from the microwire tips. Note that this system can be used for either simple infusion or push-pull perfusion. (B) Microdrive push-pull guide cannula assemblies arc implanted in two sites in a cat, but perfusion tips are not in position. (C) Construction details showing two types of "plumbing" for separating the infused solution and the extracted solution from the concentric tubes. (1) The inner cannula passes through the sealed chamber above the outflow of the outer cannula. Outflow collection is made from a separate tube passing into the chamber. (2) The outer cannula is bent, with a hole above bend. The inner cannula passes through the hole, which is then sealed with solder. Polyethylene sleeves can be adjusted to determine the depth of the cannula. (D) Field of diffusion to recording sites of perfused or infused substances. (From McGinty *et al.,* 1982.)

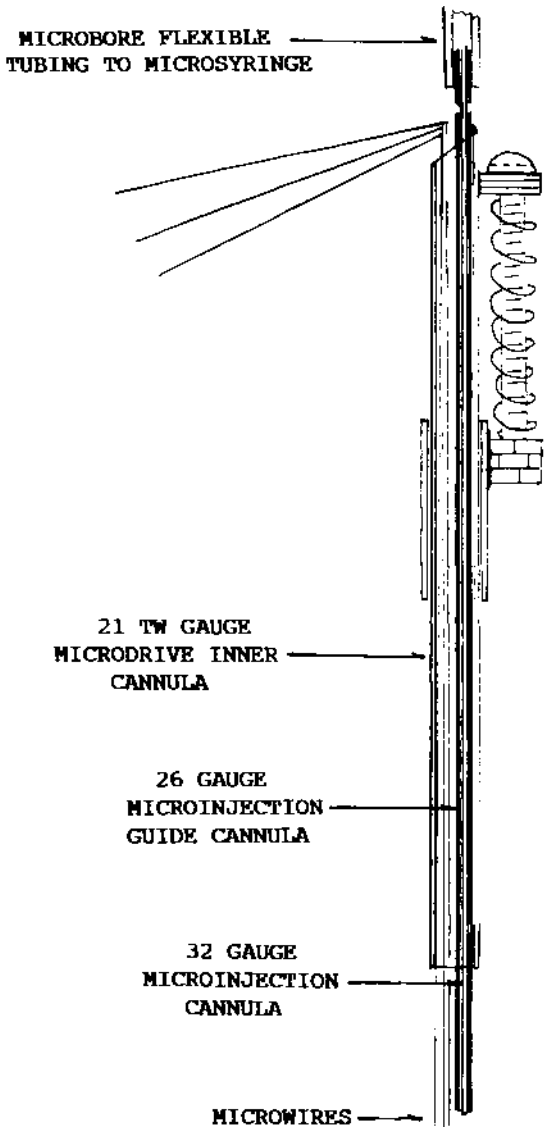


FIG. 7. Method for microinfusion very close to recording sites. In this version of the microdrive, an infusion cannula and microwires pass through the same inner cannula, so that microinjections may be within 0.5 mm of the recording site.

injection volume sufficient to induce specific behaviors (Fig. 6). If the purpose is to approximate the goals of an iontophoretic injection study (i.e., application of a very small amount of chemical very close to the recording site), then the variation shown in Fig. 7 can be used. In this case, the microinjection cannula passes within the same guide as the microwires. However, injection volumes must be very small (—0.05 /ul), and injections must be made slowly to avoid displacement of tissue away from the recording site, thereby disrupting the recording.

Finally, the need to make multiple passes through a site may apply in some situations, as when a neuronal group has little vertical depth, so that few cells of a type can be encountered on one pass, or in extremely valuable animals in which it is essential to record the maximum number of cells. For these applications, we have devised a microdrive variation for making multiple passes along slightly different paths through a single site. This is accomplished by making the inner cannula with a slight bend and incorporating a method for withdrawing the microwires and rotating the cannula after completion of one vertical penetration (Fig. 8).

Although the crucial value of this system is the ability to record unit activity in unrestrained animals for extended periods, a secondary virtue is the relative ease of application. No special instruments or tools or outside assistance is needed to make electrodes or microdrives. Thus, less of the experimenters' effort needs to be devoted to these technical matters.

Figure 9 shows a schematic recording and behavioral study facility including two channels of unit data as well as other polygraphic variables. Connections to the experimental subject are supported by a counterweighted boom. Microwire signals are led to selector switches that permit scanning of each wire for suitable recordings. The amplified and filtered signal is displayed on an oscilloscope, and may be stored on a tape recorder for future analysis. The window discriminator converts unit signals into standard pulses that may be detected by a laboratory computer interface or displayed on a polygraph. The integrator accumulates the pulses in a convenient time interval for additional display on the polygraph and rapid rate estimation. There are several commercial versions of this equipment.

While recording unit discharge, it is possible to perform a wide variety of experimental manipulations, including microinjections, brain or chamber temperature control, brain stimulation, control of lighting, food delivery, introduction of complex behavioral contingencies, or sleep deprivation with a treadmill (Fig. 9). Good visibility or video monitoring is essential, as it is important to observe the animal closely. Changes in unit discharge that are detected may be related to an unexpected component of behavior elicited by the experimental treatment.

In the examples described below (Section IV,B), we demonstrate certain advantages of the microwire technique in comparison to the conventional stiff fine-tipped microelectrode technique used in head-restrained animals. The microwire technique provides far superior temporal stability of recording and the capability

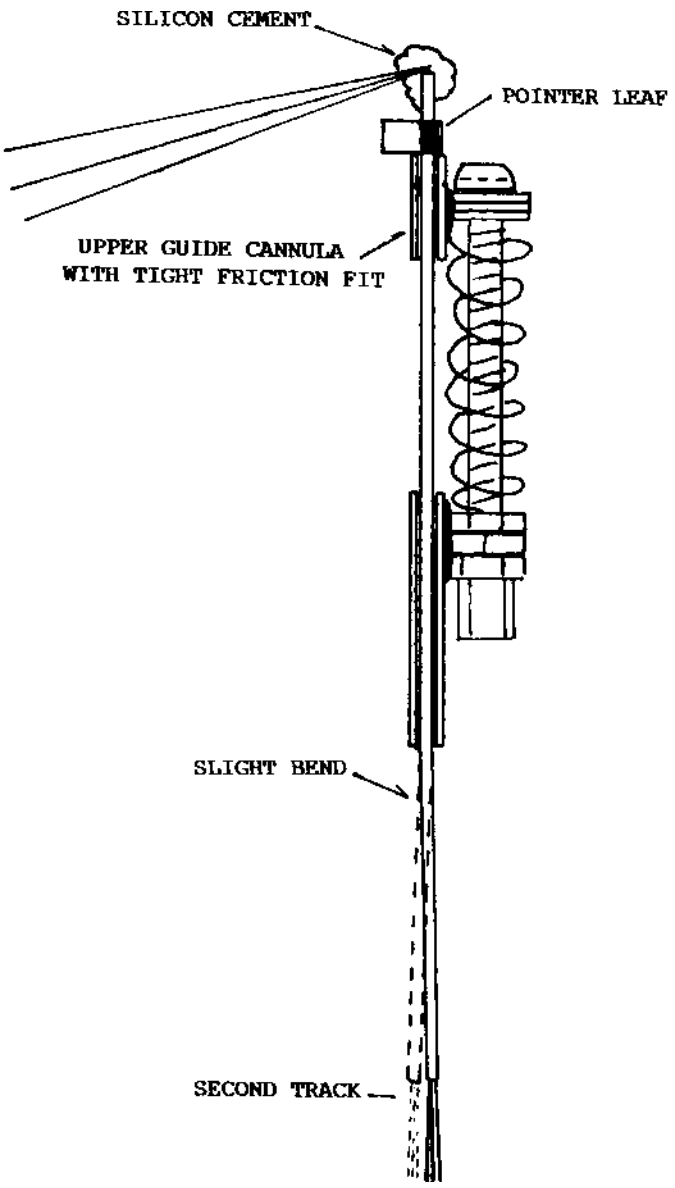
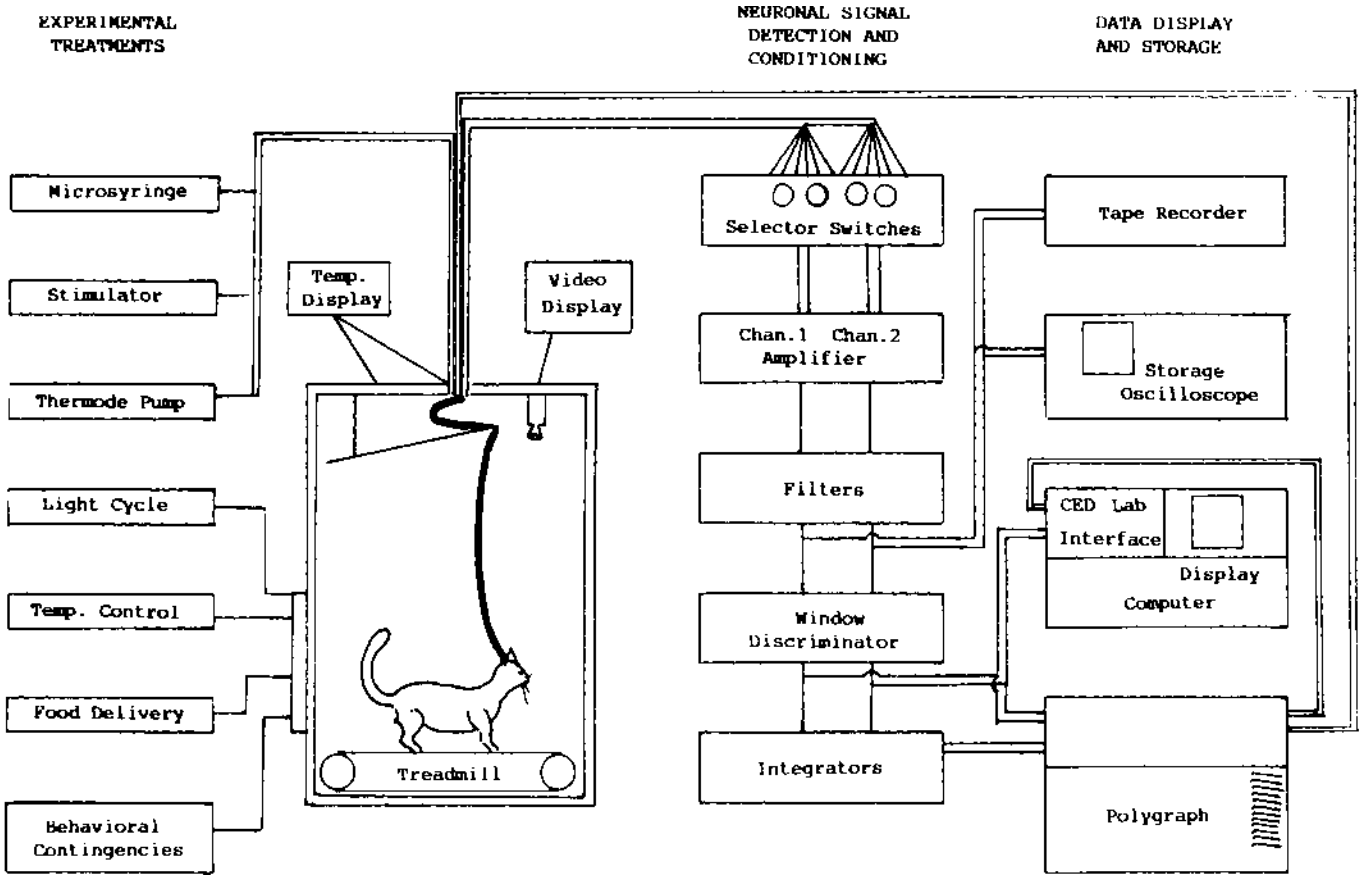


FIG. 8. Modification of the microdrive for multiple passes in one site. The lower portion of the inner cannula has a slight bend, so that when it is rotated, a new path is determined. Instead of being soldered tightly to the washers, the inner cannulae are connected by a tight friction-fitted tubing. A small metal pointer leaf, soldered to the top of the inner cannula, is used to turn the cannula and show the direction of the bend. Microwires are held in the cannula by a small glob of silicon seal. Before turning the inner cannula, the microwires are withdrawn to a position above the cannula tip. The rotating cannula tip will produce mechanical brain damage, but the diameter of the tip path may be only 1 mm.


EXPERIMENTAL TREATMENTS
NEURONAL SIGNAL DETECTION AND CONDITIONING
DATA DISPLAY AND STORAGE
Microsyringe
Stimulator
Thermode Pump
Light Cycle
Temp. Control
Food Delivery
Behavioral Contingencies
Temp. Display
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Treadmill
Selector Switches
Chan.1 Chan.2 Amplifier
Filters
Window Discriminator
Integrators
Tape Recorder
Storage Oscilloscope
CED Lab Interface
Display Computer
Polygraph

of correlating unit discharge with a wide range of behaviors. These advantages have permitted several important observations. On the other hand, the conventional stiff-electrode technique may provide superior isolation of single units, particularly in areas of high neuronal density, and better resolution of small neurons. A "stereotrode" system, in which subtle differences in wave forms recorded from adjacent microwires can be used to discriminate cells, has been developed for use in areas of high cell density (McNaughton *et a/.,* 1983). The microwire technique has been applied primarily in subcortical sites; applications in the neocortex have been demonstrated, but require further study (Lemon, 1984).

III. Overview of Sleep Mechanisms

Readers may refer to many excellent books for reviews of the complexities of sleep physiology and neurophysiology (Orem and Barnes, 1980; Borbely and Valatx, 1984; McGinty *et al.,* 1985; Wauquier *et al.,* 1985, 1989; Kryger *et al.,* 1989). Here, we summarize only the important features of sleep that are crucial to understanding the search for neural substrates of sleep mechanisms.

Sleep in mammals and birds (i.e., homeotherms) shares crucial common features; it is composed of two distinct "states," namely, slow-wave sleep (SWS), and REMS. SWS is named for the higher-amplitude lower-frequency (slow-wave) EEG patterns that identify this state under normal conditions. During REMS, the EEG is "activated" or desynchronized as it is in waking. However, REMS periods can be identified by the absence of postural muscle tone in conjunction with EEG activation. These states alternate within extended periods of sleep, REMS occurring at roughly 10- to 100-min intervals, depending primarily on the species. SWS occurs initially at sleep onset, except in neonates, which may have sleep-onset REMS. Total sleep time varies with species and is correlated with the basal metabolic rate of the species, although the functional

FIG. 9. Experimental apparatus used for unit recording and a variety of experimental manipulations. Microwire signals are conveyed in separate wires to selector switches which determine input to both amplifiers and filters. In this diagram, two unit recordings can be done simultaneously, but a three- or four-channel system may be useful. Filters are usually set to pass frequencies between 300 and 5000 Hz (— 6 db), although it may be useful to compare electroencephalographic and unit recordings from the same electrodes in some types of experiments. Switches permit the scanning of all wires for suitable recordings, as seen on the oscilloscope. Unit signals of suitable amplitude, stability, and separation from other units are detected by window discriminators, which generate pulses for computerized data analysis and for display on a polygraph. Pulse integrations are also displayed on the polygraph for rapid quantification of data. Data may also be stored on a tape recorder for subsequent analysis. We use a Cambridge Electronic Design (CED) computer interface system, which includes a variety of spike-train analysis software, but several commercial systems are available. Separate cables are used to convey additional neurophysiological or physiological signals directly to the polygraph.

significance of this correlation continues to be debated (Zepelin and Rechtschaffen, 1974). The proportion of sleep which is REMS ranges from about 6% to 46% in adult mammals and, across species, is correlated with the maturity of the species at birth and an aspect of its ecological niche: Predators and animals with secure nests have more REMS (Allison and Cicchetti, 1976). Again, these correlations are not well understood in functional terms. Sleep in neonates may consist primarily of REM (e.g., Valatx *et al,* 1964). Within SWS, there are more differentiated EEG-defined substates in higher primates and humans, a probable reflection of the elaboration of the neocortex.

Sleep in poikilothermic animals seems to be quite different from that in mammals. Although these animals may have clear sustained behavioral quiescence, the distinctive EEG features of both SWS and REMS are absent in reptiles, amphibians, and fish. The sleeplike state of poikilotherms is more dependent on environmental conditions; transitions between states are gradual. Some observers (e.g., Flanigan *et al.,* 1973) have suggested that toporlike states in reptiles and lower animals are most akin to SWS. However, the differences between poikilothermic and homeothermic sleep is striking, and it is reasonable to hypothesize that many of the specialized neuronal adaptations underlying both SWS and REMS are connected to the physiological adaptations found in homeotherms.

SWS and REMS are literally odd "bedfellows." They are very different states. SWS is associated with reduced cerebral metabolic rate, reduced (although not absent) neuronal activity in most brain regions, reduced cerebral blood flow, and a radically changed pattern of cerebral neural activity, compared to waking (see McGinty and Beahm, 1984). In contrast, REMS is characterized by an activated brain with an associated increase in cerebral metabolic activity. On the basis of neuronal discharge patterns, the brain seems as if it is awake in REMS, except for certain differences (see Section IV,D,G below). However, while upper motor neuronal systems are activated in REMS, most spinal motoneurons are paralyzed by potent hyperpolarization, producing the so-called muscle "atonia" of REMS. Although SWS and REMS are different in most physiologically measurable ways, it is important to remember that these two states provide continuity of behavioral sleep and reduced sensory responsiveness. The neurophysiological correlates of the behavioral and psychophysiological similarities of SWS and REMS are not established.

There is an important distinction between "phasic" and "tonic" components of REMS. Thus, although REMS is a sustained tonic state, as identified by EEG and motor criteria (i.e., atonia), there are superimposed phasic events such as REMs, twitches, and changes in heart rate. REMS-deprived animals have more phasic events in their recovery sleep, suggesting that the expression of phasic events is a critical regulated feature of this state. While phasic events tend to be "made up" in recovery sleep, total REMS time is not. The best-studied phasic

The XXXXXXX of REMS and may have a role in triggering this state. PGO waves are also coincident with individual eye movements of REMS. PGO waves have been studied extensively because they are frequent, stereotyped, and discrete events susceptible to neurophysiological analysis and because they have been localized in structures that are hypothesized to be critical for generation of REMS. Transection studies have shown that regions caudal to the midpontine nucleus reticularis pontis oralis are not required for the generation of PGO activity released by reserpine (Laurent *et al.,* 1974). Spontaneous PGO waves in association with a REMS-like state persist in the forebrain of undrugged chronically maintained animals with transections at the pontomedullary junction (Siegel *et al.,* 1984). Cytotoxic lesions placed within the dorsolateral pontine region between these transections eliminate PGO spikes or greatly reduce their amplitude (Webster and Jones, 1988). Stimulation of the dorsolateral pons triggers PGO spikes with a latency similar to the latency difference between the spontaneous and lateral geniculate nucleus PGO waves (Sakai *etal.,* 1976). This evidence indicates that pontine mechanisms are the critical substrate for PGO wave generation.

A PGO-like potential can be elicited in the alert wakefulness by intense stimulation (Bowker and Morrison, 1976; Wu *et al.,* 1989), indicating that the potential is related to alerting or startle. Since the brain in REMS is in many ways similar to the waking brain, it has been hypothesized that the presence of PGO waves in REMS represents an endogenous activation of the startle network (Morrison and Bowker, 1975; Morrison, 1979).

The word "state" is sometimes applied to SWS and REMS. A "state" is defined as a physiological condition which is identified by a constellation of parameters, rather than by one particular parameter. Indeed, it has not been possible to find a single physiological parameter that unambiguously defines either SWS or REMS under all conditions. Key features of a given state may occur in the absence of the state, or the state may occur without the key features (see McGinty and Siegel, 1983). For example, characteristics of the cortical or thalamic EEG normally define SWS; the words "slow wave" are derived from the normal EEG correlates of the state. However, neonatal and neodecorticate animals appear to have the quiet sleep state in the absence of EEG synchrony. After treatment with muscarinic blocking agents such as atropine, the EEG may exhibit normal sleeplike patterns in a behaviorally awake animal (e.g., Szymusiak *et al.,* 1990a). Similarly, after lesions of a specific area in the dorsal pons, cats may have what appears to be fully developed REMS, but without exhibiting the usual motoneuron paralysis, indicating that the atonia or REMS is not sufficient to define REMS under all conditions.

Thus, none of the usual correlates of either SWS or REMS can be used alone to identify the state under all conditions. Under certain experimental conditions, they may appear independently of the sleep-waking state in which they normally appear. This implies that the neural generators of each of these correlates of sleep states are mediated by separate neuronal circuits; they may function in the absence of the parent state. An important concept is that the neural basis of both SWS and REMS must include processes that recruit these separate component generators to the symphony of conditions that constitute the state. In summary, sleep state mechanisms would seem to include the sum of their correlated parts, plus the mechanism that recruits or integrates the partially independent component networks.

Another aspect of sleep state physiology is the sensitivity of state patterns to certain physiological variables (McGinty, 1985). SWS is augmented while REMS is reduced at mildly elevated body temperatures, at elevated blood pressure, and in a hypoxic atmosphere (hypoxia with hypocapnia). The occurrence of SWS under these conditions tends to reduce the physiological stress by reducing temperature, blood pressure, and metabolism. Thus, sleep appears to participate in overall homeostatic processes. Further, sleep also modulates neuroendocrine function. SWS is known to gate or trigger the release of growth hormone and prolactin. Sleep onset inhibits the release of corticotropin and thyroid-stimulating hormone. These hormonal changes are appropriate for a condition of rest (McGinty and Beahm, 1984).

Also, sleep directly alters physiological regulatory systems. For example, sleep onset changes the gain of the chemoreceptor control of ventilation and induces heat-loss mechanisms, reflecting a lowered thermoregulatory set point (see McGinty and Beahm, 1984). These physiological changes affect the sleep control mechanisms, as noted above. Thus, sleep both changes and is changed by physiological variables, establishing a feedback control loop. For example, sleep onset lowers the regulated temperature set point, which would establish a heat-loss condition (relative heat load), until temperature falls to the sleep-associated set point. This heat load may facilitate SWS. Similar regulatory loops may apply to blood gas and blood pressure regulation. The existence of such feedback loops implies that each sequential state must establish an equilibrium on the basis of these sleep state-homeostatic physiological interactions.

States undergo sequential changes in a cyclic pattern. Within sustained sleep, SWS and REMS alternate in nonrandom patterns. In humans and monkeys, the cycle is quite predictable. For example, in humans, REMS reappears at 90- to 100-min intervals. In other mammals, the REMS-REMS interval is quite irregular, but has clear modal values. Sleep-waking is also cyclic, particularly in strongly circadian animals. However, elimination of circadian rhythmicity by making lesions of the hypothalamic circadian "clock" pacemaker in the suprachiasmatic nucleus changes only the 24-hr patterning of sleep and has little

effect on the amount of sleep or short-term SWS-REMS architecture (see McGinty and Beahm, 1984). Thus, the 24-hr cycle affects the timing, but not the generation, of SWS and REMS.

The point of this brief review is to provide perspective on the issue of searching for the neural basis of sleep. In this search, we must be aware of the possibilities of misinterpretation of data because:

1. An experimental manipulation may change one of the correlates of sleep, rather than the state itself. For example, muscarinic blockers do not induce sleep, even though they induce a sleeplike EEG (see McGinty and Beahm, 1984).
2. Experimental manipulations may alter physiological variables, modifying the equilibrium between sleep and physiology. This, in turn, could cause an apparent state instability unrelated to state-generating mechanisms.
3. Neurophysiological events that appear to be correlated with a sleep state may only be correlated with a physiological concomitant of sleep and could be seen in the absence of the state if the appropriate physiological variable is changed to the critical level.
4. Sleep is easily prevented by discomfort or stress, or any stimulus that may increase the arousal level of the animal. REMS is blocked by a very wide variety of drugs and chemical agents; in many cases, this may only reflect physiological stress or disequilibrium, rather than a specific action of the agent in question. For example, the suppression of REMS by the a-adrenoreceptor antagonist phentolamine is secondary to the hypothermia caused by the drug, and can be prevented simply by maintaining the animal's body temperature (Kent *et al.,* 1987).
5. Sleep and waking states do not appear in an all-or-none fashion. Scoring manuals describe the defining characteristics of REMS and SWS in order to facilitate communication among investigators. However, it is naive to interpret such definitions as indicating an abrupt event susceptible to latency analysis in the same way that evoked unit responses are. For example, REMS onset can be defined as the point at which the first burst of REMs occur in conjunction with EEG desynchrony and muscle atonia. If one takes this definition too literally, it is tempting to see any antecedent change in neuronal activity as causing this state change. In fact, there is a regular progression of physiological changes preceding "scoring manual-defined" REMS onset. For example, EEG desynchrony usually precedes the first REM, typically by 10-30 sec in the cat. EEG desynchrony itself is always preceded by the appearance of PGO spikes 30-60 sec earlier. These spikes show a regular reduction in amplitude and an increase in frequency prior to the appearance of EEG desynchrony. It would be erroneous to argue that EEG desynchrony "causes" the REMs, or that PGO spikes "cause" EEG desynchrony or the REM sleep state itself. However, precisely the same argument has been made for neurons showing activity changes before the manual-defined onset of REMS and waking (Hobson *et al.,* 1974). Therefore, it is important to

appreciate the continuous nature of the spontaneous transitions into and out of sleep states in evaluating the role of any neuronal group in state transitions. Of course, events that occur earlier, rather than later, in a transition are more likely to be related to the control of the transition, but timing itself is not a sufficient argument.

This brings us to a second issue with regard to neural changes preceding physiological states. One can use statistical techniques to compare the latency of antecedent changes in various cell groups. The approach is to find the group with the earliest change, and then make the argument that this group has a causal role in the state transition. This approach is preferable to that of attributing a causal role to any neuron with activity changes prior to scoring manual-defined sleep state onsets. However, it can be equally misleading. The point at which a change in neuronal activity becomes statistically significant is determined by two variables. One is to point at which that cell group receives excitatory or inhibitory input, or autochthonously generates a change in activity; this is the variable of interest. The second variable is the amount of noise in the system. This may be a function of other inputs arising in the system, the noise caused by the recording technique, the aspect of spike discharge parameters used (e.g., simple rate, modal spike interval per unit of time, the duration of the sample used, the "baseline" firing rate of the cell in question, use of membrane potential measurements versus extracellular measurements, and use of cell pairs versus single-cell measurements). It is often difficult to separate the variable of interest from the noise involved in the measurement process. Latency measures are useful in the analysis of events occurring on the order of milliseconds, in which only a few synapses can be involved. However, latencies are usually quite unconvincing when analyzing state transitions occurring over a period of tens of seconds or minutes.

IV. Evaluation of Hypotheses Concerning Sleep Control

A. LOCALIZATION OF REMS MECHANISMS

Studies by French investigator Michel Jouvet and colleagues using chronically maintained animals with brain stem transections suggested that a neural generation of the REMS-like state could be localized in the lower brain stem. One can cut through the midbrain in the coronal plane, so as to separate the caudal brain stem from the diencephalon and telencephalic structures. Animals with such lesions manifest all of the brain stem signs of REMS that can be observed *caudal to the cut.* Atonia, REMs, and PGO spike bursts, as well as a REMS-like activation of reticular formation units occur in a regular ultradian rhythm (Jouvet, 1962; Villablanca, 1966). Therefore, one may conclude that structures rostral to

the midbrain are not required for REMS, and that structures caudal to the midbrain contain neurons that are sufficient to generate REMS. Transection at the junction of the spinal cord and the medulla does not prevent all of the signs of REMS from occurring rostral to the cut (Adey *et al.,* 1968; Puizillout *et al.,* 1974). Thus, spinal mechanisms are not essential for the generation of REMS. From the above, we may conclude that structures caudal to the midbrain and rostral to the spinal cord are necessary for REMS.

This technique has been carried one step further by transecting between the medulla and the pons and maintaining the animals for extended periods to allow the fullest possible recovery from the transection (Siegel *et al.,* 1986). As was the case with midbrain sections, the brain regions rostral and caudal to the cut produce independent physiologically defined states. The medulla cycles regularly between an activated and a quiescent state. The activated state is characterized by high levels of muscle tone, identical to those seen in active waking, and by accelerated respiration and heart rate. The quiescent state is characterized by lower levels of muscle tone, resembling those seen in SWS, and by slow regular respiration. Periods of muscle atonia are not seen. Unit activity, recorded with our microwire technique, in the medial medulla during the quiescent state of the medullary animal resembles that seen in this region in SWS in the intact animal (i.e., it is slow and regular) (Fig. 10). Unit activity increases, but remains regular during the activated state. Thus, the respiratory regularity, the low level of muscle tone, and the unit activity during the quiescent state are similar to the conditions of SWS. The increased muscle tone and the unit activity during phasic arousal are similar to the conditions of waking. Periods of increased unit activity alternate in a regular ultradian rhythm with periods of quiescence with tonic regular discharge. Periods of irregular unit discharge, like those usually seen in normal REMS, do not occur. This indicates that the medulla and the spinal cord, disconnected from rostral structures, show spontaneous variations in levels of arousal and SWS-like states, but do not show the medullary signs of REMS. Structures caudal to the pons are not sufficient to generate REMS.

A very different picture is seen in rostral structures after transection between the pons and the medulla (Siegel *et al.,* 1984). Three states can be distinguished rostral to the transection. The first is a synchronized state without PGO spikes, resembling SWS. Thus, both the medulla and the forebrain show independent SWS-like states after these transections. The second state is a desynchronized state without PGO spikes, resembling waking. The third state is a de-synchronized state with PGO spikes. The PGO activity occurs in irregular bursts and as isolated spikes in a manner very similar to that seen in REMS. Midbrain reticular units show irregular burst-pause patterns of discharge in conjunction with this third state (Fig. 11), as they do in REMS (Siegel, 1985).

From the above, one can see that when the pons is connected to mid- and forebrain structures, most of the defining signs of REMS are seen in these rostral

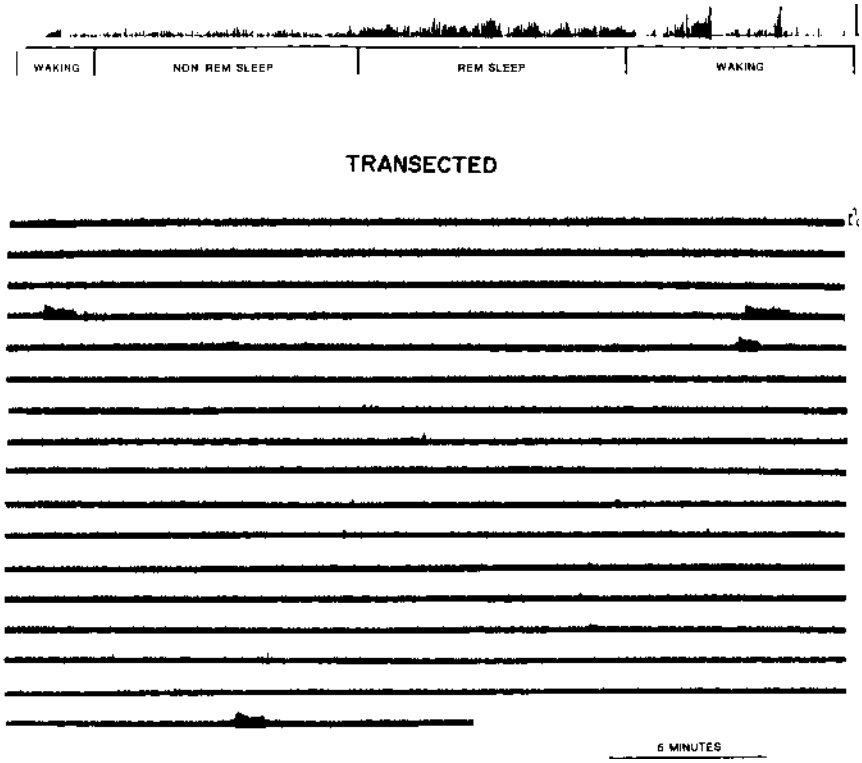


FIG. 10. Medullary reticular formation (RF) unit activity during the sleep-waking cycle in an intact cat (baseline), and after brain stem transaction at the pontomedullary junction. Tracing is the output of a digital counter resetting at 1-sec intervals. Note the long periods of accelerated and irregular RF unit discharge during waking and REMS in the intact cat. RF cells in transected cats had extremely regular discharge rates, interrupted by short periods of increased unit activity occurring in conjunction with movement arousals. Thus, unit-recording data supported the hypothesis that medullary mechanisms did not generate a REMS-like state. (From Siegel *et al.,* 1986.)

structures. When the pons is connected to the medulla and the spinal cord, as in the midbrain decerebrate animal, most of the defining signs of REMS are seen in caudal structures.

One can then transect through the middle of the pons and again ask the question "Which side has REMS?" (Siegel *et al.,* 1984, 1986). After this transection, the caudal pons and medulla shift between the aroused and quiescent states seen in the medullary animal (Siegel, 1985). No atonia or other signs of REMS are present. The rostral pons and forebrain show desynchronized waking-

like states and synchronized SWS sleeplike states. The *synchronized* states may be accompanied by PGO spikes and PGO spike bursts, resembling those seen during desynchrony in REMS and REMS transitions. However, midbrain unit activity is greatly *decreased* at these times, in contrast to the activated pattern seen in REMS in the intact animal. PGO spikes do not occur in the desynchronized state. Therefore, with this midpontine transection, we reach the limit of the transection technique. The major defining characteristics of the REMS state are absent on both sides of the transection, even in chronically maintained animals.

While the foregoing indicates that the pons is necessary for the generation of REMS-like states in both rostral and caudal structures, one may ask, "Is the pons, in and of itself, sufficient to generate the pontine aspects of REMS?" One can monitor REMS signs after transecting *both* rostrally and caudally to the pons, producing an acute isolated pons preparation (Matsuzaki, 1969). In this case, the pons continues to generate periodic episodes of REMs and PGO spikes in a pattern which, in the intact animal, is seen only in REMS. This is impressive evidence of pontine control of these basic aspects of REMS.

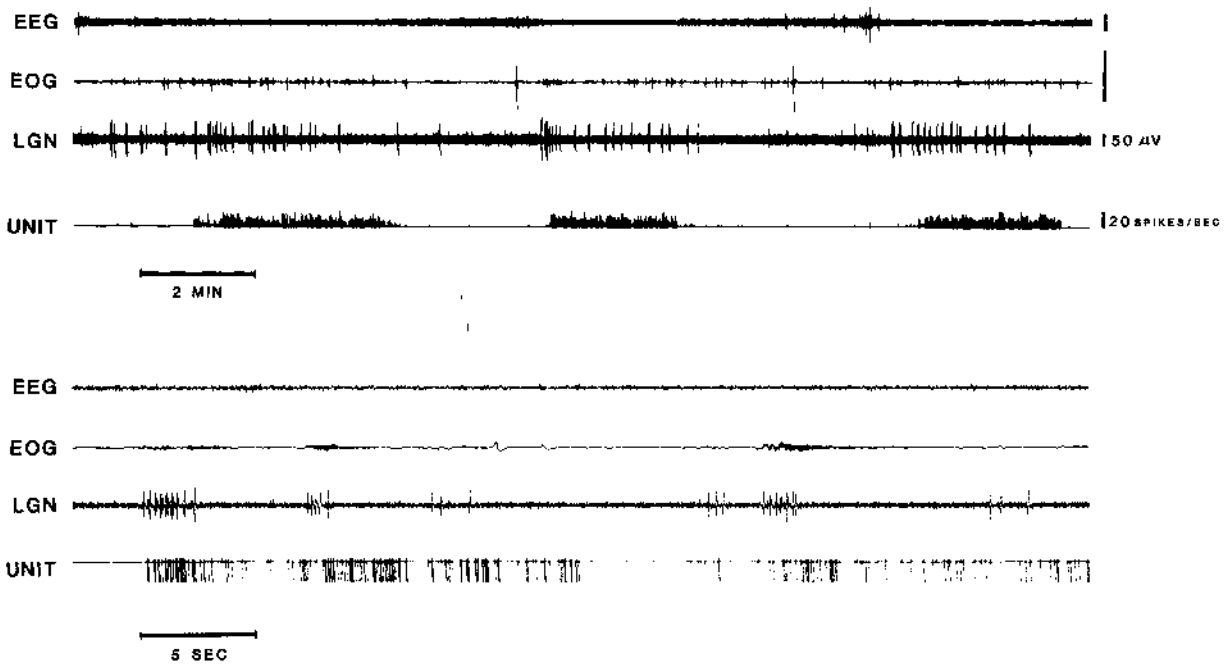
B. REMS TRIGGERING AND THE MEDIAE PONTINE RETICUEAR FORMATION (MPRF)

The next logical question was the identification of the critical cell groups for REMS generation within the pons. Hobson and colleagues hypothesized that mPRF cells were the critical neuronal elements. Cells in this area were found to have very high discharge rates in REMS, but were silent, or had relatively low discharge rates in SWS (Huttenlocher, 1961; Hobson *et al.,* 1974). In sleep-deprived cats adapted to the head restraint used during recordings, these cells have little waking activity, with waking rates comparable to those seen in SWS (Hobson *et al.,* 1974). They were also found to have a detectable increase in discharge 2 min before the "onset" of REMS (defined as the first REM burst), that is, "tonic latency."

However, in freely moving cats that were not sleep deprived, we found that virtually *all* mPRF cells discharge in waking at rates comparable to mean REMS rates (McGinty and Siegel, 1977; Siegel and McGinty, 1978; Siegel *etal.,* 1977). Discharge rates during active waking are positively correlated with REMS rates in a population of cells (Siegel *et al.,* 1979b). Figure 12 shows a continuous recording of a typical medial pontine unit during an extended period of waking and REMS. It is clear that the cells may be less active in waking than in REMS, but that during phasic episodes of activation, REMS and waking rates may be similar. During waking, periods of neuronal activation corresponded to periods of movement. Thus, the comparison of rates in different states depends on the behavior of animal during the sampling period.

POSTTRANSECTION
DAY 11

UNIT ACTIVITY DURING PGO



We devised a number of procedures for eliciting reflexes and testing with somatic, auditory, and visual stimuli. We also observed activity during spontaneous behaviors over periods averaging several hours. We quantified suspected movement relationships with a Hall-effect device [voltage induced in a conductor moving in a magnetic field (Nienhuis and Siegel, 1989)] and with photographic techniques (Siegel *et al.,* 1979a). We also devised a technique by which the animal could help us deduce the behavioral relationship. We reasoned that if the animal was reinforced for increasing discharge in the neuron of interest, it would increase the behaviors correlated with neuronal discharge. In this sense, the animal is required to determine the behavioral correlates of discharge in order to get reinforcement. The experimenter merely needs to observe the behavior that emerges and see whether it is the same as that observed during spontaneous firing and in response to appropriate stimuli. We accomplished this by reinforcing cats with hypothalamic stimulation for increasing discharge rate in these cells. We found that the animal quickly learned to produce the movement associated with maximal cell discharge repeatedly, much as an animal might learn to bar-press for reinforcement (Breedlove *et al.,* 1979). At the same time, with the photographic method, we could analyze the movement produced by the animal to obtain reinforcement (Fig. 13).

We found that most medial reticular cells discharge maximally in conjunction with a directionally specific movement of the head, neck, eyes, limbs, or facial musculature (Siegel and Tomaszewski, 1983). The most common correlate of increased discharge rate of cells in the mPRF is ipsilateral head movement. Such movements could be expected to occur in a variety of situations, especially those in which intense or noxious stimuli are applied.

One would expect centrally commanded skeletal movements (whose expression in REMS is blocked by motoneuron hyperpolarization) to accompany the REM bursts and muscle twitches of REMS. Thus, the apparent "selectivity" of discharge for REMS in cats recorded during head restraint can be seen as a consequence of the reduction in *waking* motor activity caused by the restraint, rather than indicating any essential role in REMS control for these cells (Siegel, 1979a). This conclusion is buttressed by studies showing no disruption of REMS

FIG;. 11. Midbrain reticular formation (RF) unit recordings with a cat with a pontomedullary transcction. Unit activity during cortical electroencephalographic (EEG) desynchrony with PGO waves, or "spikes," shown at two different polygraph speeds. In the upper (slow) trace (see time calibration), the unit channel displays output of a digital counter resetting a 1-sec intervals. In the lower (faster) trace, individual unit potential signals trigger the deflections shown on the polygraph record. In contrast to the unit patterns recorded below the transection (Fig. 10), midbrain units showed sustained accelerated irregular discharge patterns like those normally seen in REMS, in conjunction with PGO waves. Thus, unit data supported the hypothesis that a REMS-like state could be generated by structures above the medullary RF. EOG, Electrooculogram; LGN, EEG patterns of the lateral geniculate nucleus, demonstrating PGO waves.

when cytotoxins were injected into this region. Even though this manipulation totally removed all giant mPRF cells, REMS was not disturbed, appearing in normal amounts within 24 hr of the lesion (Sastre *et al.,* 1981; Drucker-Colin and Pedraza, 1983). However, such lesions do produce a persistent motor deficit, ipsilateral head movements being permanently impaired (Suzuki *et aL,* 1989). Conversely, chemical stimulation of this region produces a sustained contraction of the ipsilateral neck musculature (Suzuki *et aL,* 1989). Anatomical and physiological data indicating monosynaptic projections from this region to spinal motoneurons innervating neck muscles are consistent with our behavioral data (see Siegel, 1979a).

Thus, anatomical, stimulation, recording, and lesion data all agree in implicating mPRF cells in motor control. This finding, dependent on our ability to observe unit activity under normal behavioral conditions, has important implications not only for controversies over the role of mPRF cells in REMS control, but for literally several hundred previous studies (Siegel, 1979a). Early concepts of the reticular formation as a diffuse arousal system had led to many studies of unit activity in this area. Typically, these studies used anesthesia, or physical restraint. In the few cases in which the unrestrained preparation was utilized, little or no systematic observation of the animal was used. Investigators routinely found patterns of unit activity consistent with their arousal-related hypotheses. Thus, cells thought be to specifically related to "alertness," pain, reward anticipation, or attention were found virtually everywhere in the medial reticular formation. However, the assumption that the underlying relationship was to arousal-related processes blinded investigators to simpler relationships. Also lost in the literature were the underlying contradictions in much of this work. For example, one group of investigators reported a majority of mPRF cells discharged selectively in relation to pain in the same region that another group found cells discharging selectively in relation to REMS, while a third group found the majority of cells discharging in relation to reward (Siegel, 1979a). Only with the observation of these cells in the normally behaving animals could the underlying movement relationship be observed, explaining the unaddressed contradiction of how a single cell could be active in so many different behavioral situations.

In summary, since mPRF neuronal activity is not selective for REMS, it is unlikely to be critical for REMS generation. Detailed studies of these neurons

FIG. 12. Continuous film record of amplified medial pontine reticular formation (mPRF) unit recording in waking (~11 min) and REMS (~4 min) in the freely moving animal. Note the variability of mPRF unit discharge in both states. Discharge bursts during waking were associated with specific movements. This is consistent with several types of evidence relating mPRF neuronal discharge to the control of movement (see text). We have hypothesized that the variability of REMS discharge reflects the internally programmed motor activity of this state. Also illustrated are quality and stability of unit recordings using the microwire method. (From Siegel *et aL,* 1977.)

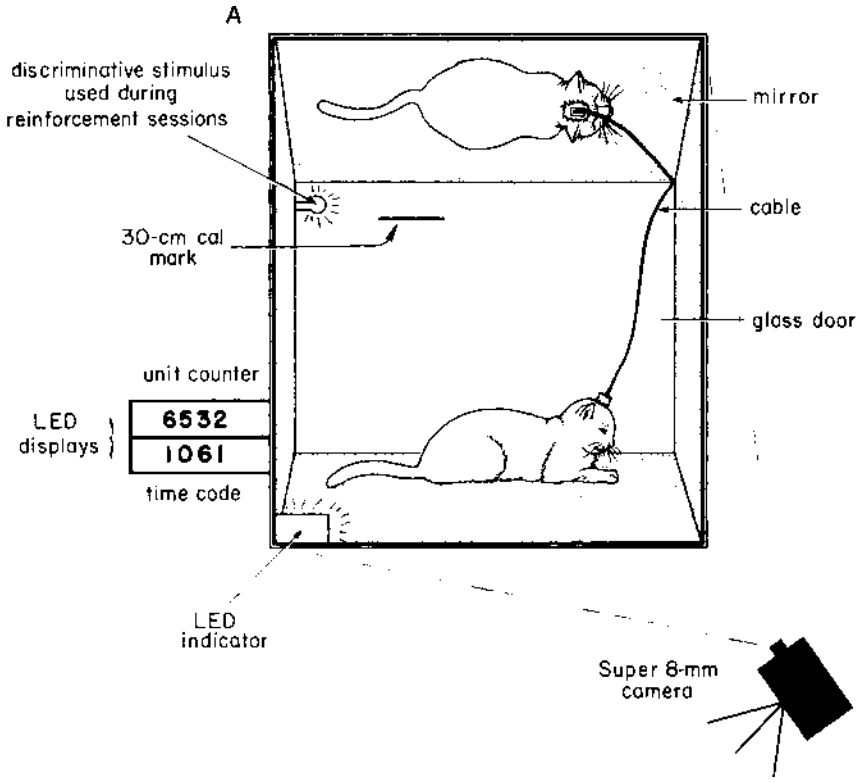


FIG. 13. (A) Arrangement of equipment used for the analysis of unit discharge-movement relationships. A film or video camera records the animal's movements, along with an LED display and incremental counter, which show each unit discharge. A second counter displays a time code, which is also written on the polygraph. (From Siegel *et al.,* 1979a.) (B) Unit discharge of a medial pontine reticular formation cell in which increased discharge was reinforced by hypothalamic stimulation. Each point represents total discharge in 2 min. B, Baseline (no reinforcement) periods: R.

showed that they participated in the regulation of movement. Subsequent studies showed that lesions of the mPRF had little or no effect on REMS, although subtle effects on motor control could be demonstrated. Thus, lack of selectivity established in unit-recording studies proved to be consistent with other findings. The original finding of REMS-selective discharge was an artifact resulting from restriction of movement. Reliance on correlational data to suggest a causal hypothesis proved to be incorrect. The tonic latency criterion that supported the REMS generation hypothesis was not sufficient, because it is difficult to define the initial event in a cascade of events lasting minutes, and in which there is little

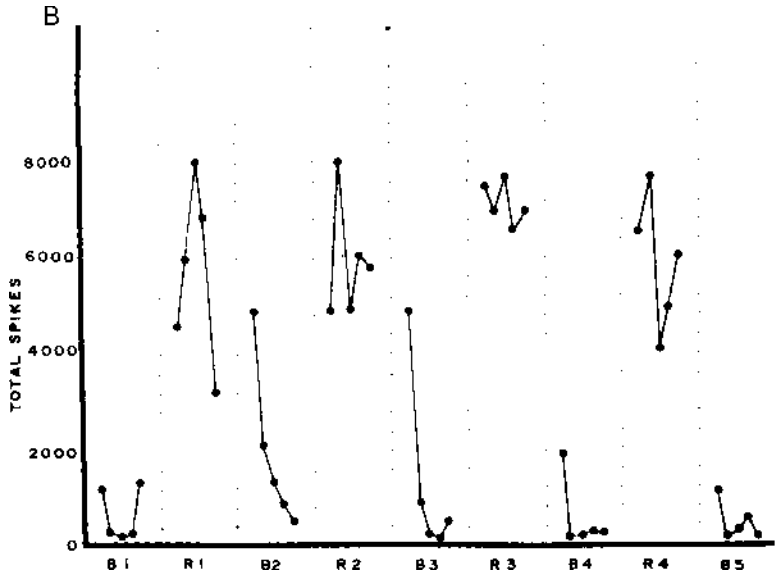


FIG 13 *(com.)*
reinforcement periods. The cat was presented with a discriminative stimulus signaling reinforcement periods. Note the increased discharge during reinforcement periods and successive "extinction" curves in the intervening baseline periods. Higher discharge rates were accompanied by repetitive directionally specific head movements, as determined by the photographic method shown in (A). (From Breedlove *el al.,* 1979.)

understanding of the critical onset processes. Below, we describe several additional cell types that appear to be part of a sustained cascade leading to REMS.

C. DORSOLATERAL PONTINE AND MEDIAL MEDULLARY RETICULAR CELLS

A different picture has emerged in unit recordings from the lateral pontine and medial medullary reticular formation. Many cells in these regions have discharge profiles similar to those seen in medial pontine regions and may have similar involvement in motor control. However, these areas also contain a population of cells that discharge at a high rate throughout REMS and have little or no activity in SWS (Sakai, 1980). Figure 14 shows an example from our laboratories. In waking, these cells are generally silent, even during vigorous movement. However, some are active during head lowering and related postural changes involving reductions in tone in a number of muscles (Siegel *et al.,* 1979b). The pontine REMS-on cells are distributed throughout the region implicated by lesion studies in REMS control (Siegel, 1989; Sakai, 1980; Shiromani *et al.,* 1987). This

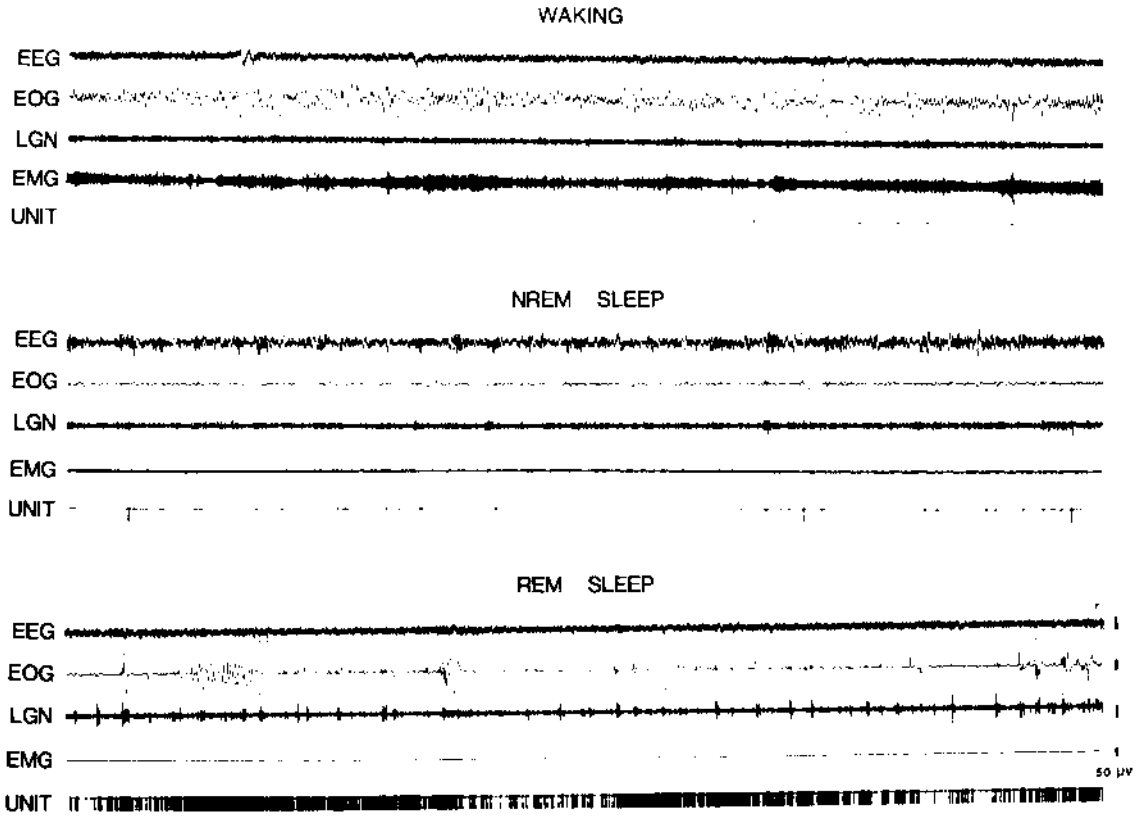


FIG. 14. Example of REMS-on cell recorded in the dorsolatcral pons (B. N. Mallick and J. M. Siegel, unpublished observations). EEG, cortical electroencephalogram; EOG, electroculogram; LGN, EEG patterns of the lateral geniculate nucleus, demonstrating PGO waves; EMG, clcctromyogram.

distribution is significant, since it indicates that the critical lesion does not merely interrupt fibers of passage in the more ventrolateral portion of the critical area, but also removes the somas of cells that are selectively active in REM sleep. A recent paper (Shiromani *et al.,* 1988) reported that the REMS-on cells in this lateral region are not cholinergic, although it is likely that they are cholinoceptive. Aminergic cells are also not present in this region. Thus, the transmitter used by these cells remains unknown. The medullary REMS-on cells are located in an area receiving projections from the pontine REMS-on region (Sakai *et al.,* 1979).

D. NORADRENERGIC CELLS OE THE LOCUS COERUEEUS COMPEEX

Noraclrenergic cells [norepinephrine-containing, herein referred to as NE] in the locus coeruleus complex have relatively regular discharge patterns in waking, in contrast to the burst-pause discharge pattern seen in medial reticular neurons. During the initial stages of SWS, these cells slow slightly. During the "transition" to REMS (defined in the cat as the time at which PGO spikes begin appearing), discharge in both serotoninergic (serotonin-containing, herein reterred to as 5-HT) and NE cells slows dramatically. During REMS, these cells have their lowest discharge rates, and many are completely silent (McGinty and Sakai, 1973; Aston-Jones and Bloom, 1981; Hobson *et al.,* 1975; Saito *et al.,* 1977). The significance of these unit activity patterns for REMS control and for the "function" of REMS is unclear. The slowing of discharge in these cells in SWS argues against the hypothesis that increased release of NE triggers REMS. The minimal discharge rate of these cells in REMS also argues against the hypothesis that NE maintains REMS. However, the complete absence of discharge in REMS does suggest that these cells may have a role in gating, inhibiting, or disinhibiting some aspect of REMS. The observation that pharmacological depletion of NE results in increased REMS amounts supports such a hypothesis (e.g., Hartmann *et al.,* 1971). In several models of REMS control, NE neurons are included in a neuronal network in which there are inhibitory interactions between REMS-excitatory and -inhibitory neuronal types (Hobson *et al.,* 1975; Sakai, 1985; Pompeiano and Valentinuzzi, 1976). However, lesions that destroy most of the NE cell group of the locus coeruleus have little effect on the amount of REMS (Jones *et al.,* 1977). Thus, the role of NE neurons in REMS is not understood. Siegel and Rogawski (1988) extended an earlier hypothesis (Hartmann, 1970) that the critical functional mechanism involves REMS effects on NE activity, rather than an effect of NE activity on REMS. They have advanced the hypothesis that, by suppressing NE discharge, REMS results in up-regulation of receptor responses, with benefits in waking function.

, CHOLINERGIC NEURONS

a lety of pharmacological and brain microinjection studies have supported .ole for acetylcholine (ACh) in regulation of REMS. REMS is suppressed by systemic or pontine microinjection of muscarinic blocking agents such as atro-pine (but see below) and by the ACh synthesis inhibitor hemicholinium (e.g., Domino *et al.,* 1968; Hazra, 1970). REMS-onset triggering may be accelerated in the decerebrate cat and in sleeping humans by intravenous administration of physostigmine (Pompeiano and Hoshino, 1976; Sitaram *et al.,* 1976). Direct microinjection of cholinergic agonists, particularly carbachol, or an acetylcho-linesterase inhibitor, neostigmine, into the dorsomedial pons elicits sustained episodes of REMS (George *et al.*, 1964, and many others). Cholinergic receptors have been identified in this site, and fibers to this region have been traced to identified cholinergic cellular groups in the dorsolateral pontomesencephalic tegmentum, the pedunculopontine tegmental (PPT) area, and the laterodorsal tegmental nuclei, using double-labeling techniques (Shiromani *et al.,* 1988). Further, in the cat, some PPT neurons discharge in discrete bursts beginning 30-40 msec before PGO waves, and these cells project to the thalamus, where PGO waves are most prominent (Sakai arid Jouvet, 1980). PGO waves invariably precede REMS episodes in the cat. On the basis of such evidence, it has been proposed that cholinergic activation is an essential mechanism for triggering REMS, and that REMS is regulated by the interaction of cholinergic and aminergic (NE and 5-HT) neurons. These two classes of neurons are hypothesized to be mutually inhibitory, such that NE- and 5-HT-containing neurons must be off and acetylcholine-containing neurons must be active to produce REMS (Hobson *et* «/., 1986).

The observation that often prolonged periods of REMS may be elicited by microinjection of the cholinergic agonist carbachol into the medial or central dorsal pontine tegmentum, often with very short latency, has been confirmed repeatedly since its first observation by George *et al.* (1964). On the other hand, as reviewed above, mPRF neurons are not selectively active in REMS. We wondered whether carbachol was acting via the mechanism that had been assumed (i.e., by activating mPRF neurons) or whether there was something unique or unexpected about the response of pontine tegmental neurons to the local application of carbachol. To answer this question, we utilized the method of microwire recording adjacent to the site of carbachol microinjection (Shiromani and McGinty, 1986).

This study showed that carbachol activated only slightly over one-quarter of mPRF neurons, all of which were also activated in spontaneous REMS periods. Twice as many cells exhibited *decreased* discharge during REM-like periods elicited by carbachol, although these same cells increased discharge in spontaneous REMS. Thus, while carbachol had a stimulatory effect on some cells,

responses were not identical to the activity patterns found in normal spontaneous REMS periods. In another study, we recorded mPRF neuronal activity adjacent to microinjection of a protein synthesis inhibitor, chloramphenicol. Application of chloramphenicol reduced the frequency of REMS periods and suppressed discharge of adjacent mPRF neurons, particularly at the time of abortive REMS transitions (Drucker-Colin et al., 1982). Injections of an inactive analog of chloramphenicol had no such effects. Thus, these studies supported the hypothesis that PRF activation could participate in the triggering of REMS, even if this was only a facilitatory role. On the other hand, as shown by recording unit activity during carbachol injections, massive mPRF activation is not essential for triggering REMS.

Experimental studies of cholinergic PPT neurons could also be used to assess hypotheses concerning the role of ACh in REMS. We may ask whether such PPT neurons are invariably active before and during REMS and whether they are selectively active in REMS? Researchers have also been concerned with the technical problem of identifying ACh-containing neurons, since the PPT area is not absolutely homogeneous. However, it has been reported that, within the PPT area, at least 80% of neurons projecting to the thalamus are either cholinergic or NE. The latter can be recognized by their characteristic REM-off discharge pattern, which is similar to that of 5-HT-containing neurons. Therefore, cholinergic neurons might be tentatively identified as those PPT neurons projecting to thalamus that are not REMS-off types. However, it should be noted that the interpretation that PPT neurons projecting to thalamus are all cholinergic has been questioned on the basis of in vitro neurophysiological studies. Recent studies of PPT slice preparations have suggested that neurons within the PPT area that have the membrane conductance bursting mechanisms (low-threshold slow depolarizations crowned by bursts) are not cholinergic (Kang and Katai, 1990). in vivo studies show that bursting PGO-related neurons may project to the thalamus. However, these constitute only about 5% of PPT neurons (Steriade et al., 199()b).

There have been several studies of PPT neurons identified by antidromic activation from the thalamus, including some cells that exhibit the PGO burst discharge pattern. Figure 15 illustrates our results, which are in general agreement with those of others (McGinty and Szymusiak, 1988b). In our study, we distinguished groups of cells exhibiting PGO-related bursts (some of which were identified by antidromic activation), other antidromically activated cells, and cells showing generally similar discharge patterns, but which could not be activated antidromically. All of these cells were somewhat alike in that they were characterized by low discharge rates, even during peak activity. All showed very low discharge rates without bursting in SWS. Two of these groups showed increased discharge in the pre-REMS period (S-PGO), and all showed increased discharge in active REMS compared to SWS. However, these cells were not

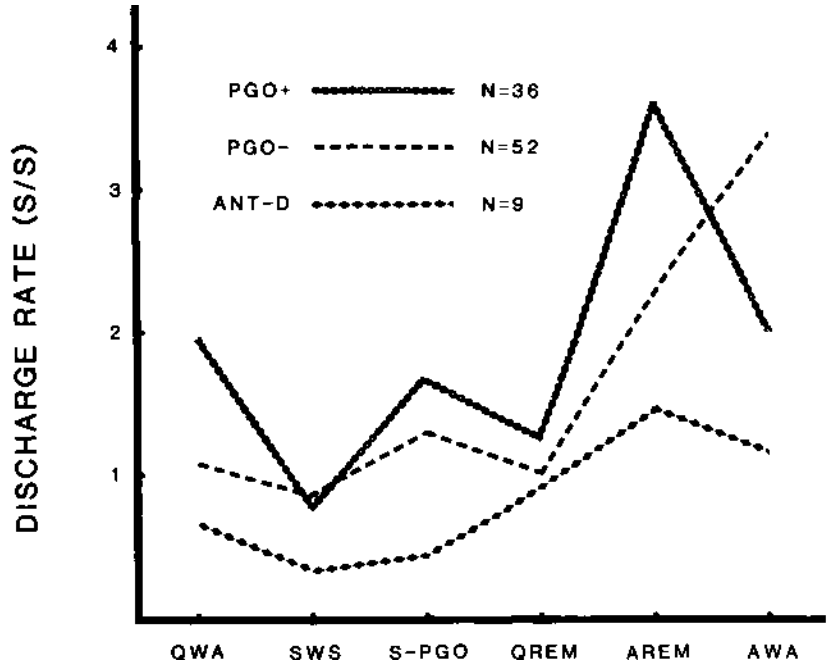


FIG. 15. Discharge rates in spikes per second (S/S) of cells recorded in the pedunculopontine tegmental (PPT) area in the cat during sleep and wakefulness. Separate rate profiles are provided for cells with discharge tightly coupled to PGO waves (PGO+) or not (PGO —), and for cells that could be antidromically activated from the thalamus (ANT-D). Rates were determined for active waking (AWA, far right), active REMS (AREM), quiet REMS (QREM), SWS with PGO waves (S-PGO), SWS, and quiet waking (QWA). All cell types exhibited relatively slow discharge in all states. PGO + cells had higher rates in AREM than in AWA, but AWA rates were higher than QREM rates. In other cell types. AWA rates were equal or higher than those in REMS states. Discharge was low in SWS. These data do not provide strong support for the hypothesis of unique involvement in the control of both active and quiet REMS by PPT neurons, but suggest a more complicated mechanism. (From D. McGinty and R. Szymusiak, unpublished observations.)

selectively active in REMS. Active waking rates were higher than pre-REMS rates in each of these groups. Although PGO-related cells had higher rates in active REMS than in active wake, rates in quiet REMS and pre-REMS were comparable to awake rates. The only distinctive event related to REMS was the burst discharge; this seemed to be absent or rare during waking.

Stenade *et al.* (1990a,b) have completed more detailed studies in head-restrained cats in which they define a number PPT neuronal types. These include slowly discharging PGO-related "sluggish"-burst neurons, tonically discharging high-frequency PGO-related burst neurons, very slowly bursting PGO-related

neurons. PGO-on single-spike neurons, PGO-off neurons, and post-PGO-on neurons. They hypothesized that the sluggish-burst neurons correspond to the PGO-related neurons described previously; they argued that these are probably not cholinergic on the basis of the *in vitro* studies summarized above. The other PGO-related neuronal types may mediate the cholinergic facilitation of REMS and PGO waves, while the sluggish-burst neurons would convey the specific PGO event to the thalamus. The Steriade *el al.* papers described a detailed model by which PPT neurons, in conjunction with other brain stem mechanisms, could facilitate REMS, PGO waves, and EEG activation.

These findings would be consistent with the following conclusions: (1) Since aminergic and cholinergic neurons both exhibit near-maximal rates during active waking, they cannot be regulated primary by mutually inhibitory interactions. Thus, the Hobson-McCarley model prediction (Hobson *el al.,* 1975) is not fulfilled. (2) Cholinergic activity does not occur selectively in REMS, but may also occur during waking. While such activity must be one facilitatory influence on REMS, it may not itself determine REMS onset. Indeed, there is some recent evidence that this is the case. We have found that the inhibitory effects of atropine on REMS seen at normal laboratory temperatures (23°C) are greatly attenuated in a warm ambient temperature (30°C) (Szymusiak *el al.*, 1990b). Thus, while there is little doubt that cholinergic input to the dorsal PRF may elicit REMS, this response may normally depend on some additional factors. One component may be the PGO sluggish-burst-cell input, and inhibitory gating by norepinephrine and 5-HT is also involved. Additional still unidentified dorsal pontine neuronal elements, including REMS-on cells could also be essential elements in a complex network that regulates REMS. Such a conclusion would be consistent with the survival of REMS after restricted lesions of ACh neuronal areas. However, after more extensive dorsal pontine lesions involving both cholinergic areas and adjacent areas, REMS may be suppressed for long periods (Webster and Jones, 1988).

F. NEURONAL BASIS OF SWS

Recent studies (see Steriade and Elinas, 1988) have strongly supported the hypothesis that the generation of synchronized or desynchronized EEG patterns depends on two primary mechanisms, which we summarize briefly here. First, individual neurons in many central nervous system (CNS) sites may generate sleeplike or waking-like neuronal discharge patterns on the basis of different voltage-dependent conductances. In particular, high-amplitude slow waves in the membrane potential with characteristic brief high-frequency bursts of neuronal discharge may be triggered by brief neuronal excitation during a hyperpolarized state. The slow depolarization results from activation of a so-called low-threshold C A^2 +-dependent conductance. This slow depolarization is blocked if the membrane resting potential is relatively depolarized. In the latter case, excitatory

inputs result, not in bursts, but in more sustained spike trains characteristic of the waking state. Whether or not thalamic neurons exhibit slow waves depends on the resting level of membrane polarization. This resting polarization level is hypothesized to be regulated by cholinergic and serotoninergic inputs from the brain stem. A second mechanism appears to account for the synchronization of neuronal pools associated with SWS. Intrathalamic circuits originating in the reticular nucleus and possibly the midline, intralaminar, and dorsomedial nuclei initiate feedforward and feedback inhibition of thalamic relay neurons to produce the critical hyperpolarized state and to "synchronize" groups of cells. Thus, with respect to the issue of the control of EEG synchronization in SWS (one aspect of the sleep process), it is necessary to understand how the resting potential of thalamic neurons is controlled.

Since behavioral sleep may occur after ablation of the thalamus, it is likely that the regulation of EEG control originates outside of the thalamus. The mechanisms regulating SWS would be expected to modulate motor and autonomic aspects of the state, in conjunction with EEG synchrony. Several SWS-regulating mechanisms have been proposed, including the medullary reticular formation, the serotoninergic system, and the basal forebrain-preoptic hypothalamus.

G. 5-HT AND SWS

A brilliant initial attempt to integrate sleep mechanisms with "chemical neurophysiology" was Jouvet's (1967) set of hypotheses involving 5-HT and SWS, on the one hand, and NE cells of the locus coeruleus and REMS, on the other. The development of the histofluorescent method and the anatomical localization of the 5-HT- and NE-containing nuclear groups had just been developed by Falck *et al.* (1962). Jouvet and co-workers found that, in the cat, lesions of the 5-HT-containing raphe nuclei, or chemical blockade of 5-HT synthesis, caused insomnia, as well as the release of PGO waves in waking (see Jouvet, 1967). Other pharmacological data were consistent with these findings. They interpreted these findings to mean that 5-HT was an essential element in the generation of SWS.

Our investigation of this issue was based on the premise that the actions of 5-HT would depend on the release of transmitter, as triggered by nerve impulse flow. Thus, knowing when 5-HT-containing neurons were active would indicate how this putative transmitter would influence sleep-waking states. The identification of 5-HT-containing neurons was based on their localization in the relatively homogeneous dorsal raphe nuclei and the distinct regular slow discharge pattern of these cells, as discovered in anesthetized animals. The identification of 5-HT-containing neurons in this fashion was confirmed in several subsequent studies (e.g., Trulson, 1985).

In contradiction to the prediction of the Jouvet theory, we found that 5-HT-containing neurons showed an approximately 50% reduction in discharge during

SWS compared to waking, and a dramatic near-total suppression of discharge during REMS (McGinty et al., 1973; McGinty and Harper, 1976) (Fig. 16). Further, many, but not all, of these neurons exhibited phasic suppression of discharge prior to PGO waves during the pre-REMS period. The latter finding was consistent with observations that drugs depleting the brain of 5-HT resulted in the release of PGO waves during waking and SWS, when they are normally absent (e.g.. Dement et al., 1972). Thus, release of 5-HT may normally inhibit the occurrence of PGO waves. The finding of SWS-related neuronal slowing, with further slowing in REMS, was extended by Jacobs and co-workers (e.g., Heym et al., 1982) to the other brain stem raphe nuclei that comprise the sites of 5-HT-containing neurons.

The inference that the release of 5-HT was reduced during sleep was subsequently confirmed by examining the release of 5-HT in target sites. This was accomplished by both perfusion and electrochemical detection techniques (Trulson, 1985). In addition, in the rat, 5-HT turnover is lowest during the light phase of the circadian day, when sleep is predominant (Agren et al., 1986). Thus, the unit-recording findings are consistent with results from most other methods. There is one great advantage to unit recording, namely, the possibility of determining specific close temporal correlations between serotoninergic function and behavior. In the case of 5-HT-containing neurons, the awake behavioral data do not support a role for 5-HT in specific temporally discrete functions, including movements or responses to sensory stimuli. Only weak modulation in relation to movement or sensory input has been observed (Fig. 17). This finding can be contrasted with studies of mPRF neurons, discussed above (Section IV,B), that revealed a close relationship between neuronal discharge and specific behaviors.

We believe that these studies illustrate the great usefulness of chronic unit recording in the evaluation of theoretical hypotheses. In this case, lesion data were misleading. In the face of this and other evidence, Jouvet et al. (1989) have modified his theory to a version in which the release of 5-HT during waking may cause an indirect facilitation of SWS through actions in the hypothalamus. This modification of the theory would rationalize the findings of insomnia after 5-HT depletion and unit-recording studies. However, specific mechanistic details of this model or direct evidence for SWS facilitation by waking release of 5-HT have not been presented. An alternative view is that disruption of 5-HT-dependent processes resulted in some as yet unspecified physiological instability that interfered with sleep. The latter explanation would be consistent with the finding that, with sustained depletion of 5-HT, sleep returns to near-normality (Dement et al., 1972). Cats may adapt to the physiological instability that initially disrupts sleep.

We introduced this article by noting that neuronal unit recording could be used both to test hypotheses and to suggest the nature of neural coding. For the latter application, we may consider what actions of 5-HT are indicated by the depres-

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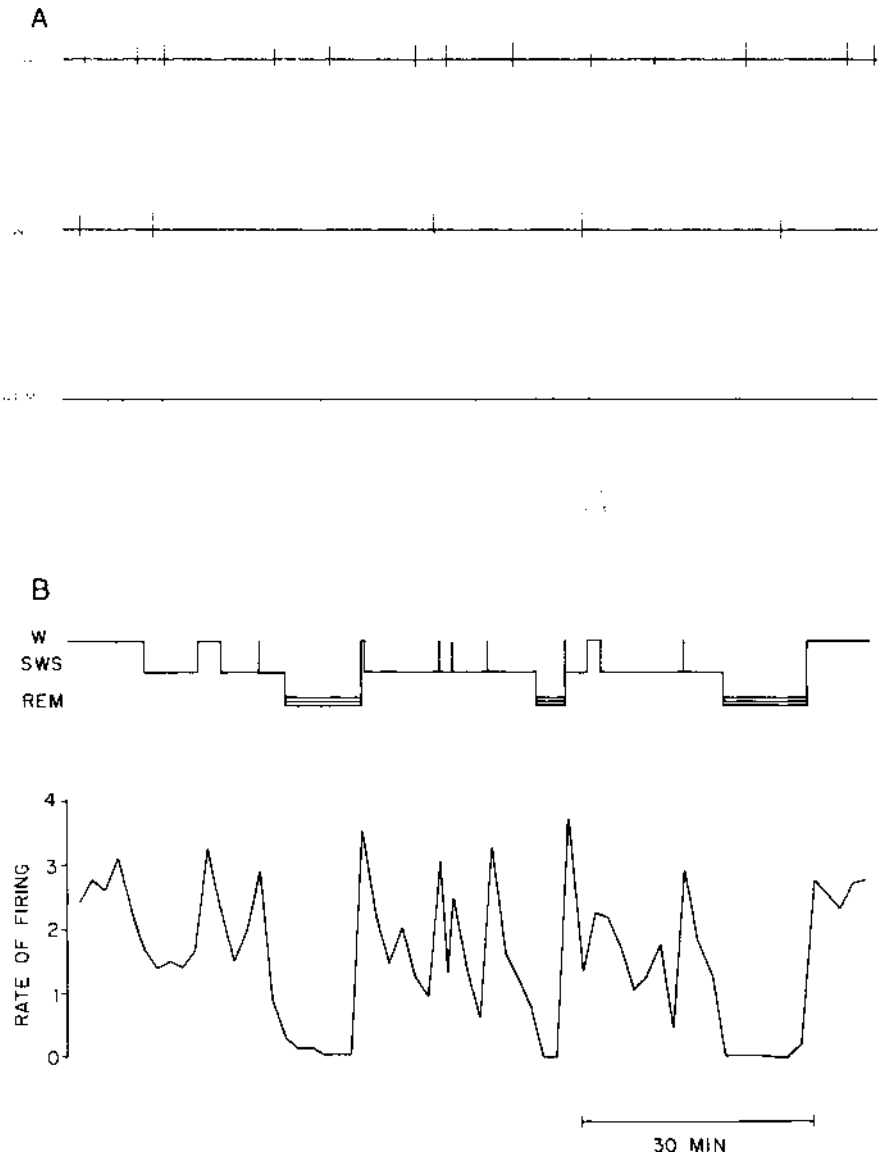


FIG. 16. (A) Oscilloscopic trace of amplified recording of dorsal raphe unit discharge in the cat. These neurons exhibit relatively regular discharge during waking (W), much slower discharge during SWS, and virtual silence during REMS. (From McGinty and Harper, 1976.) (B) Continuous record of dorsal raphe unit discharge during several waking-SWS-REMS cycles. Discharge is consistently stopped during REMS periods and elevated during waking, even during transient waking episodes. SWS discharge rates are intermediate. (From McGinty and Siegel, 1977.)

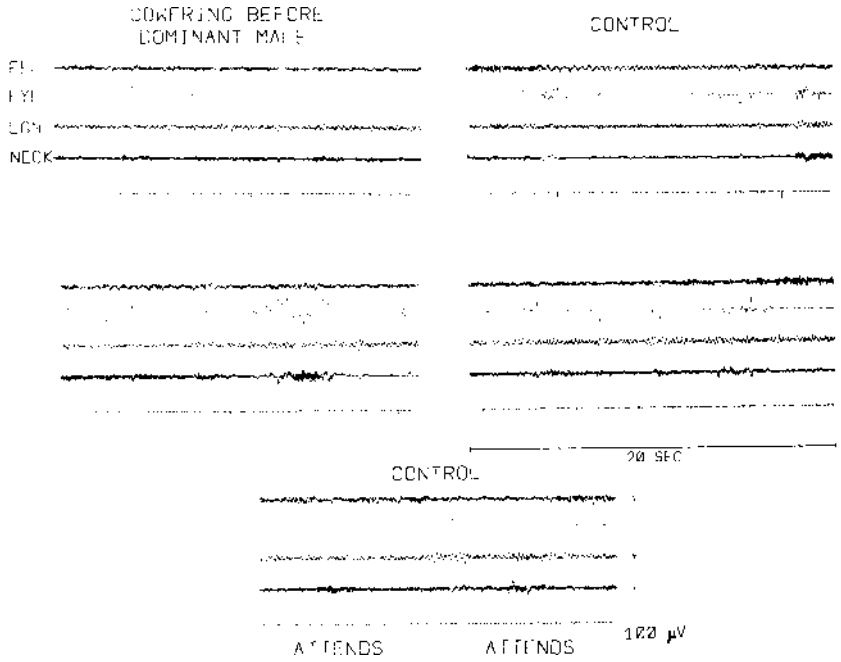


FIG. 17. An example of dorsal raphe unit during different waking behaviors. (Top) Samples on the left were obtained as the cat cowered in fearful submission and hissed in response to the presence of a dominant male cat, while control samples on the right were recorded during relaxed waking periods with eye movements. There may have been slightly increased unit discharge during intense arousal, but changes were much smaller than those occurring during each sleep cycle. (Bottom) There was no indication of altered discharge when the cat attended to a novel stimulus. EEg, Cortical electroencephalogram; EYE, electro-oculogram; EGN, lateral geniculate nucleus. (From McGinty *et al.,* 1973.)

sion of raphe unit discharge during sleep, particularly REMS. During waking, dorsal raphe discharge is unchanged by a wide variety of behavioral manipulations (McGinty *et al.,* 1973), so the sleep-related events are the most quantitatively significant naturally occurring changes in the release of 5-HT. Trulson *et al.* (1981) suggested that raphe unit discharge could be related to the central control of overall motor excitation. In this model, raphe discharge suppression during REMS is coupled with the atonia of REMS. They found that cats having pontine lesions resulting in REMS *without* atonia showed less reduction in dorsal raphe unit discharge in REMS. While this concept may have merit, the proposed 5-HT-motor tone relationship is not consistent under a wide range of conditions. Certainly, there is a weak relationship between motor activity and raphe unit discharge during waking behavior.

The functional significance of the release of 5-HT and its suppression during

sleep continues to present a challenge to neuroscientists. The unusual features of the serotoninergic neuronal system may offer a guide to future experiments (see McGinty and Szymusiak, 1988a). This system projects diffusely to all regions of the CNS and make contacts with blood vessels as well as neurons. Moreover, most terminals on neurons are not conventional junctional synaptic contacts in either the cortex or the spinal cord. lontophoretic studies indicate that 5-HT has primarily neuromodulatory actions. Pharmacological manipulation of 5-HT has been shown to affect virtually every type of behavior and basic regulatory function, including neuroendocrine function. At a neurophysiological level, 5-HT is often characterized as a neuromodulator, modifying responses to other inputs. Thus, 5-HT may regulate a nonspecific aspect of the state of the brain, rather than having a specific role in sleep control. The eventual understanding of the neuromodulatory role of 5-HT may tell us something about the nature of the state of the brain during sleep, rather than how sleep is controlled.

H. BASAL FOREBRAIN AND SWS

A hypothesis that basal forebrain (BF)-preoptic area-anterior hypothalamic (POAH) neuronal mechanisms facilitated SWS was first proposed on the basis of analysis of human encephalitic neuropathology, which sometimes produced insomnia as a result (von Economo, 1930). Subsequently, it was shown that insomnia could be produced in experimental animals with BF electrolytic or mechanical lesions (e.g., Nauta, 1946). Recently, we showed that cell-selective neurotoxin-induced lesions could have this effect (Szymusiak and McGinty, 1986a). Conversely, stimulation of this area using electrical, chemical, or thermal stimuli may induce sleep, as well as inhibit activity of the midbrain reticular areas thought to be involved in EEG arousal (e.g., Benedek *et al.,* 1982; Bremer, 1973).

These studies suggested that it should be possible to identify BF neuronal units, which could convey the hypnogenic output of this region. The issue of neuronal identification was particularly important, since the BF region, including the POAH, is extremely complex and plays a role in a wide variety of behavioral, neuroendocrine, and autonomic functions. Recently, much interest has focused on the forebrain cholinergic cells localized in the magnocellular BF, including the diagonal bands of Broca, the lateral POAH (or substantia innominata), and the subpallial region. This cortically projecting cell group was found to have degenerated in patients with Alzheimer's disease (Whitehouse *et al.,* 1982).

Using the microwire technique, we identified a diffuse subset of neurons, scattered throughout the magnocellular BF, which exhibited little or no discharge during waking and enhanced discharge during SWS and quiet REMS (Szymusiak and McGinty, 1986b). Sleep-active neurons (SANs), defined by having SWS discharge rates at least twice that of waking, were intermixed with other cell

types that exhibited increased discharge during waking and REMS, much like neurons in a variety of CNS sites. SANs constituted 24% of the total sample in one study in cats. These cells exhibited an additional property that would be expected in neurons that play a role in the control of sleep. They exhibited increased discharge before the first spindle burst of sleep, thus anticipating the state transition (Szymusiak and McGinty, 1989b) (Fig. 18). This property does not prove a role in sleep control, as state transitions are difficult to define (see above), but such a finding, in conjunction with other types of data, supports the hypothesis that SANs participate in the hypnogenic role of the BF.

As noted above, the magnocellular BF contains neurons projecting to the neocortex as well as to the brain stem. In order to determine whether SANs were projection neurons, we attempted to activate these neurons antidromically by placing stimulating electrodes in the midbrain reticular formation and in the external capsule and the anterior cingulate bundle, two fiber bundles known to carry fibers to the neocortex from the BF. Antidromic responses were identified by the usual criteria (i.e., low variability in response latency and ability to respond to high-frequency paired pulse stimulation) and by the collision test. In the collision test, a spontaneous orthodromic spike discharge is used to trigger the stimulator eliciting the putative antidromic discharge. If the triggered pulse is

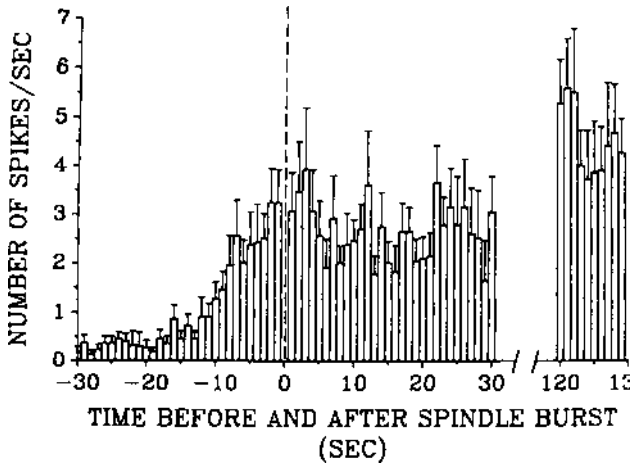


FIG. 18. Changes in basal forebrain sleep-active neuronal discharge in relationship to sleep onset, based on 22 sleep-active neurons. For each cell, transitions from alert waking to deep SWS were analyzed. The first high-amplitude spindle burst at sleep onset is indicated at time zero (vertical dashed line). Bars indicate the mean (±SEM) number of spikes for each second during the 30 sec before and after the spindle burst, and during 10 sec of sustained SWS 120 sec after sleep onset. These neurons showed increased discharge beginning 15-20 sec before the first spindle burst and a sustained increase in discharge during SWS. (From Szymusiak and McGinty, 1990.)

given within the interval of the antidromic response latency, an antidromically conducted pulse will "collide" with and be blocked by the refractory period produced by the orthodromic spike, and no antidromic spike will be observed. However, a pulse triggered after an interval that is slightly longer than the antidromic response latency will produce a response as usual.

About 30% of a sample of over 200 neurons recorded in the BF exhibited antidromic activation (Szymusiak and McGinty, 1989b). Both SANs and non-SANs could be driven antidromically from each of the three stimulation sites. For additional analysis, cells were divided into three groups: SANs, state-indifferent neurons (which exhibited very low discharge rates at all times), and waking-active neurons. This analysis showed that these subgroups of neurons could be discriminated on the basis of antidromic response latency. SANs and state-indifferent neurons invariably exhibited antidromic response latencies greater than 5 msec, averaging between 12 and 18 msec, depending on the stimulation site (Fig. 19). Waking-active neurons had antidromic response latencies from the same stimulation sites that were lower than 5 msec.

This study suggests that the population of BF projection neurons may consist of at least two subgroups with different conduction velocities and different state-related discharge profiles. It is plausible that these subgroups also utilize different neurotransmitters (Fisher *et al.,* 1988). Since we are studying cortically projecting neurons in the magnocellular BF, it is very likely that one group includes the cholinergic neurons found in these areas. Some evidence supports the hypothesis that the waking-active neurons are cholinergic (Szymusiak and McGinty, 1990): (1) In our study, waking-active neurons tended to be located more dorsally, where cholinergic neurons are more numerous in the cat. (2) ACh is released in the cortex in EEG-activated states (Phillis, 1968). (3) EEG activation associated with waking is blocked by the muscarinic receptor-blocking agent atropine (e.g., Szymusiak and McGinty, 1990). (4) At the cortical and thalamic cellular levels, ACh has depolarizing effects (McCormick, 1989).

While there is strong evidence linking ACh release, and therefore waking-active neuronal discharge, to activated EEG states, the chemical identity of SANs remains uncertain. There are reports that GABAergic neurons are also localized in the magnocellular BF, and it is plausible that such an inhibitory agent would convey sleep-inducing output. However, definitive evidence for this hypothesis has not yet been presented. Histochemical identification of SANs is needed.

An additional study showed one mechanism by which sleep-active neurons may facilitate sleep onset (Szymusiak and McGinty, 1989a). We recorded neuronal discharge in the midbrain reticular formation (MRF), the hypothesized location of EEG-activating neurons projecting to the thalamus. We selected neurons in this area with a tonic discharge during waking. We found that single-shock stimulation in both the BF and the medial POAH suppressed MRF dis-

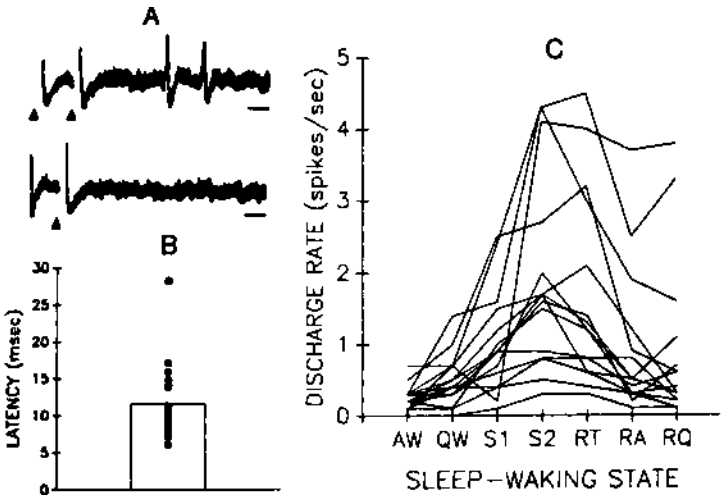
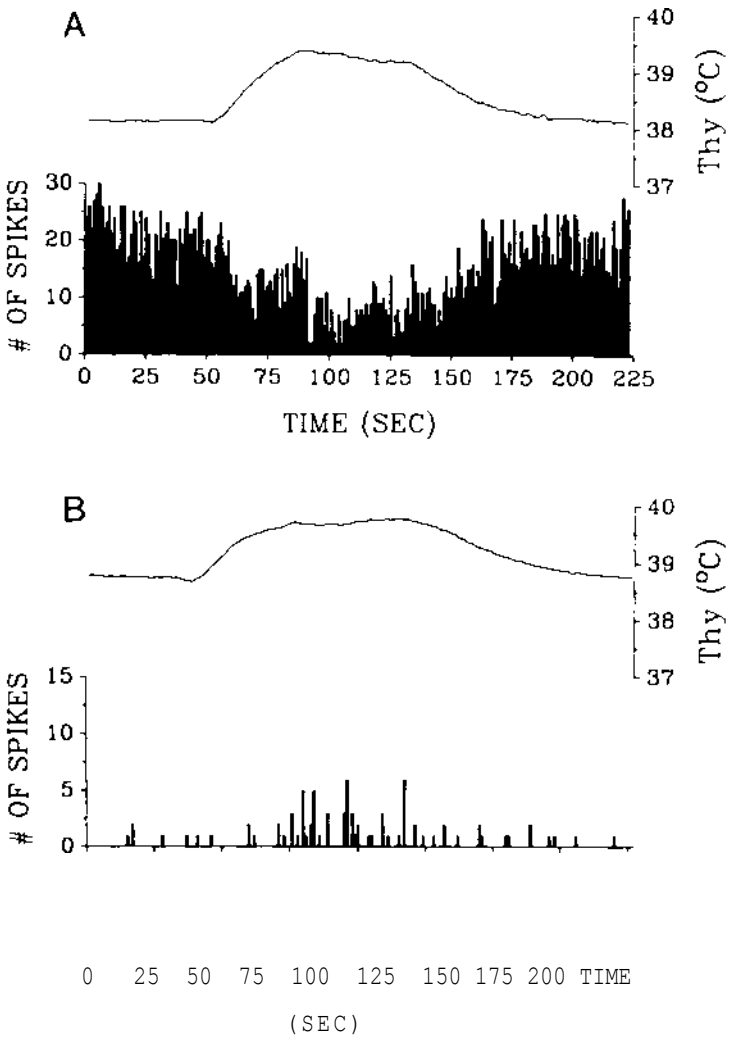


FIG. 19. Sleep-active neurons could be discriminated from neighboring cells by studies of anti-dromic activation. Shown are studies of basal forebrain (BF) neurons exhibiting antidromic activation with relatively long latency from the anterior cingulate bundle (ACB), a pathway from BF to the cerebral cortex. (A) Two standard tests for antidromic activation. (Top) BF neuronal discharges follow high-frequency (300-Hz) paired pulse stimulation (arrows) to the ACB. (Bottom) In the collision test, the ACB stimulation is triggered by a spontaneous unit discharge within the period of the response latency. The ACB stimulus fails to elicit a response, indicating "collision" with the orthodromically conducted spike. Calibration = 2 msec. (B) Mean and distribution of response latencies. (C) Sleep-waking discharge rate profiles of cells exhibiting response latencies greater than 5 msec. Slowly conducting neurons had sleep-active *(n* — 9) or state-indifferent *(n* = 6) discharge profiles. Waking-active neurons (not shown) had response latencies lower than 5 msec. For further details, see text. AW and QW, active and quiet awake, respectively; S1 and S2, Light and deep SWS, respectively; RT, transition period to REMS; RA and RQ, active and quiet REMS, respectively. (From Szymusiak and McGinty, 1989b.)

charge for 30-90 msec, sometimes preceded by a brief excitation. Thus, tonic discharge in the BF may inhibit the MRF, facilitating EEG synchronization. The chemical identity of the MRF neurons studied is not known.

Lesions of the medial POAH as well as the magnocellular BF (including the lateral POAH) have been found to result in sleep suppression (e.g., Szymusiak and McGinty, 1986a; Sallanon *et al.,* 1986). The medial POAH is not a primary site of origin of long projection neurons and may play a different role in the SWS control system. The medial POAH is the site of thermoregulatory mechanisms and contains thermosensitive neurons, including primarily warmth-sensitive neurons. A relationship between thermoregulatory mechanisms of the medial POAH and hypnogenic mechanisms was noted in recent studies. Lesions of the medial POAH produce deficits in warmth sensitivity, probably reflecting the loss of warmth-sensitive neurons that predominate in this area. After lesions, there is an



FIG,. 20. Effects of localized medial preoptic area-anterior hypothalamic (POAH) warming using a water-perfused thermode on neuronal discharge in lateral POAH-basal forebrain (BF) sites. The top traces show midline hypothalamic temperature. The bottom traces show the number of spikes in each successive 1-msec bin. (A) Example of suppression of discharge by medial warming in a lateral BF waking-active neuron. (B) Example of increased discharge in a sleep-active neuron. The cat was awake throughout the tests, so changes in discharge do not reflect passive effects of state change induced by warming. See text. (From Szymusiak and McGinty, 1990.)

elevated temperature threshold for heat-loss mechanisms such as panting. We have recently found that sleep suppression after medial POAH lesions can be reversed by placing animals in an elevated ambient temperature, suggesting that the temperature threshold for sleep as well as panting is elevated (Szymusiak *et al.,* 1991). This observation is congruent with a wide range of evidence indicat-

ing that SWS may be a thermoregulatory behavior, with a primary function of heat loss. Thus, warmth-sensitive neurons in the medial POAH are hypothesized to pfay a role in hypnogenesis as well as heat Joss, ft is well known that warming the medial POAH with a thermode may induce sleep onset (see McGinty and Szymusiak, 1990).

On the basis of these observations, we predicted that the SANs in the BF would be activated during waking by stimulation of warmth-sensitive neurons in the medial POAH. This prediction was tested by placing a water-perfused thermode with a thermocouple in the medial POAH and recording microwires in the lateral regions. A preliminary study confirmed our prediction (Fig. 20). By placing a very fine thermocouple within the micro wire bundle* we showed that there was no direct thermal stimulation at the recording sites. Therefore, the activation of SANs in the lateral POAH was due to synaptic responses mediated by afferents from the medial POAH to the BF. We also found that discharge of waking-active neurons in the BF was suppressed by medial POAH warming (Szymusiak and McGinty. 1990). We expect that future studies will provide additional information about the mechanisms by which thermoregulatory mechanisms may regulate SWS (see McGinty and Szymusiak, 1990).

V. Conclusions

We have reviewed studies of the neural control of sleep, emphasizing those which applied the measurement of neuronal discharge in behaving animals. We described studies in which unit discharge during the sleep-wake cycle was recorded in conjunction with adjacent microinjection of chemical agents, adjacent thermal stimulation, antidromic activation, and orthodromic activation. We also reported studies of neurons in animals with brain transections. In these preparations, behavior was severely limited by lesions of sensory and motor pathways. Unit discharge served as an indicator of the state of the animal which was not expressed behaviorally. Because of the stability of the recordings, the microwire method provides a capability of carrying out hypothesis-testing studies, in addition to determining discharge rate changes during sleep-waking states. These studies have supported the following general conclusions:

1. Neuronal unit-recording methods have played a key role in the assessment of hypotheses concerning the localization of both SWS and REMS control systems. On the basis of studies of three brain regions, we have concluded that neuronal unit-recording studies agree with the "consensus" about the function of local populations of neurons reached after application of a wide variety of methods. Indeed, unit-recording studies sometimes produced findings that were inconsistent with initial theories, but eventually came to be widely accepted. If unit-recording studies are done with animals studied under a range of behavioral

conditions, this method, in contrast to other approaches, does not yield misleading results. Consequently, general hypotheses may be confirmed or refuted with such methods.

2. Mechanistic features of regulatory systems may be suggested or supported by neuronal unit-recording studies. For example, the finding of REMS-off behavior in 5-HT- and NE-containing neurons prior to REMS onset was consistent with the hypothesis that these cell types may normally inhibit REMS-related phenomena, such as PGO waves. This concept has been confirmed by other methods. In the case of the BF hypnogenic system, the fact that SANs project to the cortex and the MRF indicates that the hypnogenic output of the BF may be integrated at each of these levels. The discovery of specific movement-related discharge in mPRF neurons led to a reanalysis and a new concept of the functional output of this area. These details of neural activity, like anatomical details, provide a basis for mechanistic models of function.

3. By their nature, neuronal unit-discharge studies are correlational studies, and do not, by themselves, answer questions about "causal" regulatory mechanisms. However, unit-recording studies provide temporal detail that cannot be provided by any other technique. Temporal information can be so dramatic as to imply a discrete causal relationship. When unit discharge is discretely related to events in the millisecond range, as in the movement-related discharge of medial reticular formation neurons, or the PGO-related discharge of PPT neurons, it is possible to make inferences about function. However, most neuronal groups thought to be related to sleep have not displayed such specific correlations. Certainly, correlational information can be misleading, as in the case of initial development of the Hobson-McCarley theory that mPRF neurons were executive neurons for REMS (see Section IV,B above). A similar objection may apply to any model in which temporal relationships in the range of seconds or minutes is used to suggest causal relationships. In such cases, a wide variety of intervening processes may underlie the apparent correlation. Of course, temporal ordering may be consistent with causal models, but a variety of methods are needed to support such hypotheses. For example, in the case of the putative hypnogenic neurons of the BF, described above (Section IV,H), the hypothesized function of this site was also supported by lesion studies, several types of stimulation experiments, and analysis of interactions with brain stem reticular formation and medial POAH thermoregulatory systems. Nevertheless, it will still be necessary to examine alternative hypotheses, such as the possibility that SAN discharge is only a correlate of some autonomic process associated with sleep.

4. A few neuronal types have been identified that exhibit discharge occurring selectively in a particular state. These include the slow-wave SANs of the magnocellular BF and the REMS-on neurons of the lateral dorsal pontine reticular formation. However, lesions of these BF sites do not produce irreversible sleep suppression. Rather, it appears that this site regulates sleep through interaction

with medial preoptic, posterior hypothalamic, midbrain reticular, thalamic, and possibly cortical mechanisms, as well as involving thermoregulatory and possibly other homeostatic processes. Similarly, while the control of REMS is localized within the pontine reticular formation, it is unlikely that the few REMS-selective neurons are the only generators. REMS consists of several dissociable components which must be coupled to form the integrated state. REMS control seems to involve interactions of several neuronal types, utilizing ACh, NE, and probably other as yet unidentified transmitters. However, none of these regulating agents functions selectively in REMS. The lack of selectivity of neuronal discharge for one particular sleep-waking state argues strongly that the neuronal group under study does not regulate the state in a unitary way. On the other hand, lack of selectivity does not mean that a system plays no role in a mechanism. Thus, cholinergic neurons appear to facilitate REMS, although they are not selective. There may be some "cofactorial mechanisms" that operates in conjunction with ACh to trigger REMS. Similarly, mPRF neurons are not essential for REMS. However, activation of these neurons, in conjunction with other mechanisms, may facilitate REMS or some of the phenomena of this state. Thus, neurophysiological studies would seem to confirm the hypothesis that sleep states are determined by the interactions of several neuronal systems. Similar conclusions may apply to all complex behaviors.

This raises the critical problem of choosing methods for analyzing neural mechanisms underlying complexly controlled behaviors, in which no single neuronal type is essential and coregulation by several systems is likely. We must repeat the statement in the introduction that a variety of methods are required to analyze neuronal mechanisms. However, we suggest that data from neuronal unit-recording studies would seem to be critical, since no other methods can reveal the temporal coincidence of events or dynamic changes in excitability that must underlie behavioral regulation. The microwire method we have described is simple and applicable to problems in understanding complex behavioral control systems.

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