NEUROSCIENCE

LECTURE 9: Behavioral Neuroscience

Techniques to study the neural basis of behavior.

METHOD	Description
Stereotaxic Surgery	Surgery performed using an atlas showing the location of brain areas in 3 planes of space. Used to place recording or stimulating electrodes or to destroy a particular part of the brain
Lesion Production	Destruction of a particular part of the brain. Lesions can be produced by passing electrical current (AC or DC) through an electrode or with chemicals (such as kainic acid or 6-hydroxydopamine) that destroy neurons. A lesion can also be made surgically by cutting a tract or by suction removal of part of the brain. A reversible lesion can be made by cooling (then rewarming) part of the brain or by injecting drugs (such as lidocaine).
Electrical Brain Stimulation	Stimulation of a brain area by passing electrical current through an electrode.
Microinjection	Injection of a small quantities of drug or neurotransmitter into a specific area of the brain.

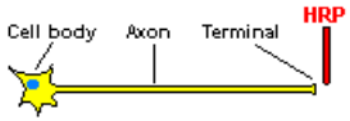
Neuroanatomy

Techniques to study the structure of the nervous system.

METHOD	Description	examples
Cell Body Staining	Coloring neurons to see individual neurons (nerve cells) or groups of neurons.	Cresyl violet stain; neutral red stain; Golgi stain Tract
Tract (Myelin) Staining	Coloring nerve fibers to see pathways.	Weil method; Weigert's myelin stain; Marchi stain
Tract Tracing	Tracing the projections from one part	Horseradish peroxidase

	of the nervous system to another part. Tracing can be retrograde (backwards) or anterograde (forwards).	(HRP) method; fluorescent microspheres; Phaseolus vulgaris-leucoagglutinin (PHA-L) method; Fluoro-Gold; Cholera B-toxin; DiI; tritiated amino acids
Immunocytochemistry	Localizing particular chemicals (neurotransmitters, proteins) within particular neurons.	
In situ hybridization	Localizing the synthesis of proteins or peptides in neurons.	
c-Fos	c-Fos is a protein product of an immediate-early gene and has been used as a marker for brain areas activated by different stimuli. To see the c-Fos, immunocytochemical techniques must be used.	
Deoxyglucose Uptake	Neurons that are active use glucose. By injecting deoxyglucose, the cells that use glucose also take up the deoxyglucose. However, the deoxyglucose is not degraded by enzymes in the neurons so it stays inside the neuron. By radioactively labeling the deoxyglucose, neuroscientist can find out what areas of the brain are active during specific behaviors or events.	

NEUROSCIENCE



Here is an example of the tract tracing method using a chemical called horseradish peroxidase (HRP). Using a small syringe, neuroscientists place HRP a small area of the brain. Neurons that project to this area will "pick up" the HRP and transport it back to the cell body (the soma). This is a form of retrograde transport because it determines what areas of the brain connect with other areas of the brain by transporting material "backwards" from the terminal of the neuron to the cell body.

Neurophysiology Techniques to understand the function of the nervous system.	
Method	**Description**
Patch Clamp Technique	Recording current flow from single ion channels of a neuron.
Intracellular Recording	Electrical recording from INSIDE of a single neuron.
Extracellular Recording	Electrical recording from outside of a single (or a few) neuron.
Mass Unit Recording	Electrical recording from outside of a group of neurons.
Evoked Potentials	Electrical activity of the brain synchronized to an event.
Electroencephalography (EEG)	Electrical activity of the brain recorded with scalp or brain electrodes.
Neuropharmacology Techniques to understand the chemistry of the nervous system.	
Method	**Description**
Microiontophoresis	Injection of small quantities of chemicals (drugs, neurotransmitter) into neural tissue by passing electrical current.

NEUROSCIENCE

Electrophysiology

INTRODUCTION

Neurons are cells specialized for the integration and propagation of electrical events. It is through such electrical activity that neurons communicate with each other as well as with muscles and other end organs. Therefore, an understanding of basic electrophysiology is fundamental to appreciating the function and dysfunctions of neurons, neural systems, and the brain.

TECHNIQUES AND MODELS TO STUDY MEMBRANE CHANNEL FUNCTION

At the cellular level, electrical activity of neurons consists of the movement of charges (ions) through neuronal surface membranes. The major charge carrying ions are sodium (Na+), potassium (K+), chloride (Cl-) and calcium (Ca2+). The surface membranes of neurons are primarily composed of lipids (resistive elements, in electrical terms) which do not allow ionic flow. Instead, these semipermeable membranes are spanned by large specialized protein aggregates that form pores or channels through the lipid membrane. There are specific channel protein assemblies (usually more than one) for each of the ionic charge carriers, as well as those for certain cations in general, that confer a semipermeable nature to the membrane. The ability of these channels to permit ion flow is determined by several factors, most prominently the electrical potential that exists across the membrane, the gradient of ions set up by membrane pumps, and the semipermeable nature of the channels, as well as by responses of receptors, guanosine triphosphate (GTP) binding proteins (termed G proteins), and second messengers to neurotransmitters and hormones. For more detail on these aspects of neuronal membrane and channel properties, (see Cholinergic Transduction, Signal Transduction Pathways for Catecholamine Receptors, and Serotonin Receptors: Signal Transduction Pathways); also see larger works by Siggins and Gruol, Hille and Shepherd.

Artificial Membrane-Channel Preparations

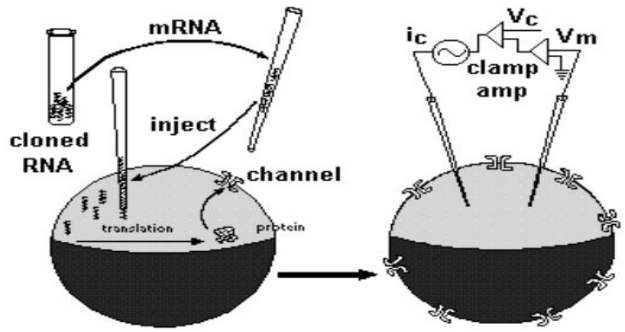
Description

There is a long history of electrophysiological studies on artificial lipid bilayer membranes that were designed in large part to determine whether bioelectric events result from membrane ion pores (channels) or transmembrane ion carriers (active transport). The results of these studies were important for substantiating the view cited above of cellular-level events involved in

bioelectric activity. Studies on pore-forming antibiotic models by Mueller and Rudin (reviewed in ref. 6) laid a strong biophysical foundation (such as the involvement of water molecules) for subsequent understanding of natural ion channels, and they were largely predictive of subsequent single-channel recordings of biological channels (see below). Later refinements of this method included insertion of natural ion channels prepared from a variety of cell types or organelles. In brief, the artificial bilayer membranes are usually made by forming a sheet of lipid (such as phosphatidylserine) across a partition with a small hole separating two aqueous compartments. A vesicle preparation (e.g., "liposomes") containing either the antibiotic protein (e.g., gramacidin), a fractionated membrane containing ion channels, or (in later studies) reconstituted vesicles with purified channels is added to one of the compartments; under the right conditions, the vesicles fuse with the lipid bilayer membrane and insert channels. Standard voltage-clamp recordings (see below) are then performed between the two compartments, and drugs and ion changes can be applied to either side of the artificial membrane.

Studies using natural membranes with artificially inserted foreign but natural receptor-channel complexes have given even more validity to the pore theory and provided another powerful model for testing pharmacological agents. The procedure here typically involves injection of a channel preparation directly into a large living cell (tolerant to insertion of large injection and recording pipettes) such as the *Xenopus laevis* oocyte or several types of cell lines. The channel source is usually either (a) vesicles prepared from fractionated channel-bearing membrane, (b) vesicles with reconstituted, purified channel (glyco)protein, or (c) channels newly synthesized by foreign DNA or RNA injected into the oocyte via large pipettes (Figure 1). Of course, the more purified the protein or DNA/RNA, the more homogenous will be the channel population eventually inserted.

Figure 1.



Schematic of oocyte expression system and voltage-clamp recording method. Left: RNA (or DNA, not shown) prepared from a cloned gene for a membrane channel is injected from a micropipette into a frog oocyte. The oocyte contains the necessary processing systems to translate this RNA (or DNA) into the specific protein, which is then incorporated into the outer membrane of the oocyte as a functional channel. Right: Voltage-clamp recording is used to examine the properties of this newly expressed channel. The large size of the oocyte allows two-electrode voltage clamp. The recording pipette transmits the membrane potential (Vm) to the clamp amplifier, which also receives a voltage command (Vc). The comparative difference between Vm and Vc is used to generate a clamping current (Ic) which is injected into the cell to bring Vm to equal Vc. In this way, Vm is "clamped" to the value of Vc; the Ic needed to maintain this Vm is equal to currents flowing across the membrane at any time-point and can be examined as a function of drug application or changes in Vc, or small changes in the composition of cloned RNA/DNA (and therefore the channel protein). These manipulations can help isolate the parts of the inserted channel responsible for each unique property of that channel (e.g., sites for antagonist or G-protein binding).

Example Studies

In terms of their great potential for dissection of the molecular mechanisms of pharmacological action, studies using these artificial, reconstituted or "cloned" channels are still in their infancy. However, there have already been too many fine examples of the use of the methods to adequately recount here. Notwithstanding, we would be remiss not to mention the elegant molecular and pharmacological studies of the gamma-aminobutyric acid (GABA) receptor–

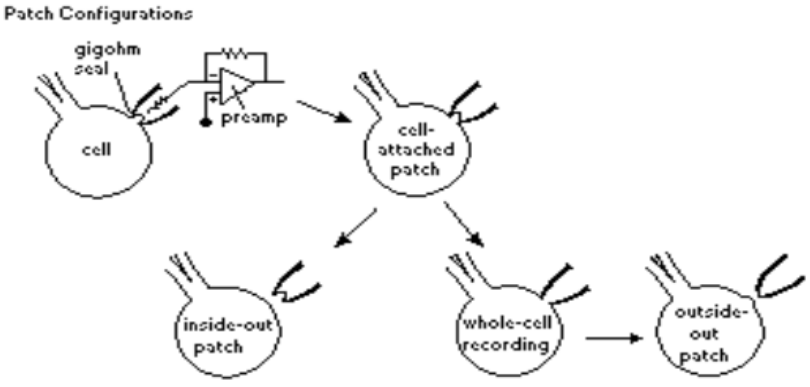
ionophore complex by Eric Barnard, Robert MacDonald, and co-workers, as well as of the various glutamate receptor-channel subtypes by the Dingledine and Heinemann groups . Moreover, recent studies of ethanol effects on brain GABA and glutamate receptor channels expressed in *Xenopus* oocytes and cell lines have provided considerable insight into these two major sites of alcohol action and provide prime examples of the potential uses of these methods for the study of the molecular mechanisms of action of psychopharmacological agents. Future studies of this type, in combination with site-directed mutagenesis, will help delineate the molecular site(s) of drug actions on a wide range of neuronal receptors.

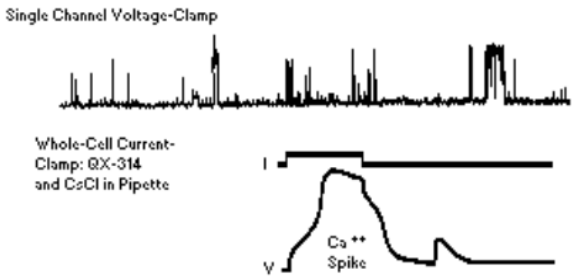
Single-Channel Patch Clamp

Description

The discovery and development, by Sakmann and Neher, of the "patch-clamp" method for recording from single-ion channels provided decisive proof of the aqueous pore theory for the origin of neuronal excitability and led to a long string of seminal studies culminating in their Nobel prize. This method originally required fabricating and fire-polishing of specific types of glass micropipettes (with large tip diameters relative to the "sharp" pipettes used in traditional intracellular recording), so that the tips could form a high-resistance (gigohm) seal when pushed onto a cultured or acutely isolated cell. The gigohm seal (and new electronic breakthroughs) essentially allowed the high current gain, low noise amplification necessary for recording the small, brief currents (under voltage-clamp conditions) passing through single ionic channels. Imagine the excitement when Sakmann and Neher saw the now familiar "box-like" currents suggesting the abrupt opening and closing of channels (Figure 2;). There were other surprises not predicted by the biophysical models, such as the rapid open-and-closed "flickering" and burst-like openings of the channels in many cases.

Figure 2.





Schematic of patch-clamp configurations and representative single-channel and whole-cell records. Upper panel: The same patch pipette and voltage-clamp amplifier setup can be used to record in four different configurations (see text for details). Single-channel recordings can be taken from three of the four configurations: cell-attached, inside-out and outside-out patches. Breaking the patch under the pipette (by suction) allows whole-cell voltage or current recording. Middle panel: A representative cell-attached patch recording (voltage-clamp mode) from a cultured CNS neuron, showing currents flowing through single ion channels. Single-channel activity appears as box-like upward events. At least two channels are present in this patch, as indicated by the different event amplitudes. Brief upward events are channel openings not fully resolved due to recording limitations (D. L. Gruol, unpublished observations). Lower panel: Representative whole-cell current-clamp recording of a hippocampal neuron in a slice preparation, recorded with a pipette containing QX-314 to block Na+ channels (fast spikes) and 140 mM CsCl to block K+ channels (G. R. Siggins, unpublished observations). This record was taken about 5 min after patching on and breaking into the cell; the Ca2+-dependent action potential (a broad depolarizing plateau without a fast Na+ component or afterhyperpolarizing potential) evoked by depolarizing current injection indicates the rapid equilibration allowed by this configuration, between the contents of the pipette and the cellular constituents. I = current, V = voltage.

The patch-clamp method can be applied in at least four configurations (figure 2), giving the technique formidable adaptability for testing the molecular mechanisms of receptors and their associated ion channels and second messengers. Three of these configurations (cell-attached patch, inside-out patch, and outside-out patch) allow study of individual ion channels under

different conditions. In the *cell-attached* configuration, after formation of the gigohm seal, recording of single channels is made without disruption of the cell membrane. In the other two single-channel preparations, the membrane patch is detached from the neuron after a gigohm seal is formed, and single-channel activity is recorded in isolation from the cell. In the *inside-out* configuration, the patch of membrane is gently pulled away from the cell, and the patch remains attached to the pipette with its cytoplasmic surface now exposed to the bathing solution. Preparation of the *outside-out* patch begins by making a whole-cell configuration (see below) whereby, after forming the gigohm seal, the membrane patch under the pipette is ruptured by applying a strong vacuum through the recording pipette. Then the pipette is gently pulled away from the cell, carrying a piece of membrane with it. The detached membrane seals over the pipette tip during this maneuver, in favorable cases forming a membrane patch in which the extracellular membrane surface is exposed to the bathing solution. The *whole-cell* configuration will be described below.

Example Studies

Once again, examples of the use of single-channel patch-clamp methods are far too numerous to itemize here. However, psychopharmacologists interested in the use of these methods in elucidating the pharmacology of NMDA, GABA, and opiate receptors may wish to consult the work of Barker and colleagues, MacDonald and colleagues, and North and co-workers . These papers contain abundant detail on the elegant methods used to record single channels and test the action of various psychopharmacological agents.

Whole-Cell Clamp

The whole-cell clamp, illustrated in figure 2, is a form of the cell-attached configuration that uses the same pipette type and gigohm seal method described above but that, by rupturing the membrane under the tip, allows recording of the "macroscopic" or summed currents flowing through all channels in the entire cellular membrane, rather than through a single channel. In this configuration, after the pipette is sealed to the membrane, another slightly stronger vacuum is applied to the pipette tip (via the tube attached to the pipette holder) to rupture the membrane under the tip without disrupting the gigohm seal or cell viability. During current-clamp recording,

successful "break-in" is signaled by a negative shift in the recorded potential (to the cell resting membrane potential) and a large reduction in the input resistance of the system (now due only to the series resistance of the pipette and the input resistance of the total cell). In this configuration, the diffusable contents of the pipette then exchange over time with those of the cell.

Example Studies

Again, there are numerous examples in the literature over the last 10 years of whole-cell patch recording of neurons in isolated systems and an ever-increasing number of whole-cell patch-clamp studies of neurons in slice preparations (see below). From a pharmacological viewpoint, the whole-cell patch studies of the MacDonald (17) and North groups (18) on second messenger and G-protein mediation or regulation of GABA, opiate, catecholamine, and somatostatin effects deserve special reference. See the chapter by Foote and Aston-Jones for details on such studies in rat locus coeruleus neurons.

Voltage and Ion-Sensitive Dyes and Resins

Description

Over the last 30 years, a variety of nonelectrophysiological (and sometimes even noninvasive) methods have been explored to measure the membrane properties and ionic constituents of excitable cells. Purely optical recording methods (without dyes) were originally applied to isolated axons (e.g., from squid or crab) and revealed changes in both light scattering and membrane birefringence during stimulus-evoked action potentials. Later studies infused a potentiometric merocyanine dye intracellularly and used signal averaging methods to show that optical absorption or fluorescence changes closely followed the time course of the action potential (19), indicating that these were useful methods for studying membrane potential by relatively noninvasive methods. The development of newer, more effective voltagesensitive dyes offer pharmacologists the opportunity to follow membrane potential changes optically, without penetration or other disruption of the neuronal membrane other than extracellular treatment with the dye.

Dyes can also be used to measure the intracellular and extracellular concentrations of certain free ions (or better, their activities). While there are now a variety of indicator dyes relatively selective for several different ions (including H+, Na+, and Cl-), by far the most research has been done with Ca2+-sensitive dyes. Metallochromatic indicator dyes like arsenazo III were

found useful for the measurement of cytosolic free Ca2+, as first tested in squid giant axons. The luminescent photoprotein aequorin also had its heyday as an indicator of intracellular free Ca2+. However, most of these methods involved the injection of the indicator into the cell, therefore requiring the study of larger neurons. The newer fluorescent probes (quin-2, fura-2, indo-1, and fluo-3) based on the Ca2+-chelator ethyleneglycol bis(aminoethyl ether)tetraacetate (EGTA) model also generally requires the use of fluorescent (ultraviolet or near-ultraviolet) illumination on relatively isolated neurons (e.g., neuronal cultures, very thin slices, or acutely isolated neurons) or isolated axon bundles. However, these newer indicators can now be loaded into neurons without injection or penetration by using their hydrolyzable esters such as acetoxymethyl (MA) ester. These esterified indicators can merely be applied extracellularly; the ester confers hydrophobicity, allowing the indicator to pass through the membrane into the cytoplasm, where the ester is removed by endogenous esterases, trapping the indicator inside. Furthermore, the recent development of scanning confocal microscopy (which can optically section a neuron without contamination by out-of-focus objects), in combination with these Ca2+ indicators, has made it possible to observe spatial or compartmental changes in intracellular free Ca2+ even in neurons within relatively thick preparations.

However, as implied above, unless a confocal microscope is available (still a rather large expense), all of these methods require a certain degree of isolation of the neurons under study. Therefore, if the chosen model is an in vivo or thick-slice preparation, the use of ion-sensitive electrodes containing ion-exchange resins, although difficult to implement, can be of considerable advantage. These electrodes can be inserted blindly into thick brain slices, or even into brain regions in vivo, to record absolute values or changes in ion activities. These electrodes achieve their ionic selectivity by virtue of the resin which generates a current flow in the electrode in proportion to the concentration of a specific ionic species. K+ and Ca2+ ion activities are the most often measured. Whereas the ion-exchange resin method is best at measuring extracellular ionic activities (because the high resistance of the resin usually requires

the use of rather large-tipped micropipettes), under the right conditions intracellular measures can also be obtained with this method .

Examples

Considerable information about neuronal and synaptic mechanisms and the effects of drugs on these mechanisms has been obtained with either the extracellular or intracellular application of ion-sensitive microelectrodes. For example, Lux, Heinemann, and colleagues have used these microelectrodes in various preparations to follow the extracellular K+ and Ca2+ levels with epileptiform activity or synaptic action via stimulation of afferent pathways.

As for the ion-sensitive fluorescent probes, fura-2 has been used to measure intracellular Ca2+ in innumerable studies. However, the ability of this indicator to measure compartmentally (spatially) distinct and time-dependent changes of intracellular calcium levels in several neuron types, with alteration of these changes by neurotransmitters, drugs (e.g., caffeine), and ion changes, is an especially exciting use of this method. This method also has great applicability for non-neuronal cells: Holliday and Gruol recently used fura-2 imaging to show that the cytokine interleukin-1b dramatically increases intracellular Ca2+ levels in cortical astrocyte cultures and also enhances the increased Ca2+ evoked by the glutamate receptor agonist quisqualate.

Advantages and Disadvantages—isolated Preparations and Patch-Clamp Analyses

The artificial membrane, reconstituted receptor/channel, and patch-clamp methods have allowed the use of isolated membrane components, isolated mRNA, foreign (non-neural) cells, and acutely isolated (enzymatically dissociated) or cultured neurons. As a result, there are several global advantages for these methods. First, these isolated preparations represent greatly simplified systems whose electrophysiological responses are not confounded by uncontrolled synaptic or hormonal inputs. Indeed, the total external and internal milieu of the cells and channels can be controlled by the experimenter, thus providing unprecedented capabilities for testing the influence of countless influences and variables. For example, the researcher can adjust the ionic compositions on either side of the membrane or channel so that the voltage differential is exactly opposite (i.e., positive on the inside surface) to that in normal cells; this facilitates greater isolation of membrane conductances involved in certain membrane and synaptic functions.

Another advantage of these isolated membranes and systems is their suitability for use of molecular and genetic techniques (described by Barondes). Thus, as implied above, one can

easily test the effects of minute modifications of the molecular structure of receptors and their associated channels, as well as the individual components (subunits) of G proteins and second messenger systems (described by Zigmond). As a result, researchers can now answer many physiological questions at the molecular level. For example, one can determine exactly where [i.e., at what codon(s) in the RNA or amino acid(s) in the protein] in the channel molecule or subunit that an antagonist or drug acts, or at what point protein phosphorylation (e.g., via protein kinases) can modulate receptor function. Thus, using combined electrophysiology and molecular biology, for the first time we can begin to make a definitive connection between structure and function at the molecular level.

The several disadvantages of these models are principally derived from the isolation process itself. Hence, these isolated preparations, because they lack normal synaptic connections with other neurons, may be functioning under conditions greatly different than normally seen in the living organism, and thus may provide answers not relevant to the "real world." In addition, one is never certain that the "extracellular" and "intracellular" media used do not constitute a totally artificial environment that would not be relevant to a living neuron in vivo. The lack of normally circulating agents such as steroids, hormones, plasma proteins, and other colloidal substances could lead to drastic changes in the function of the molecules and channels under study. Finally, this inability to examine receptor and channel function in the context of an intact functioning system may cloud the application of findings derived from these models to the behavior of living organisms.

The patch-clamp method is nearly ideal for the study of the mechanisms of drug action at the single-channel level. Some of the advantages of this method include the following: (a) Ions, toxins, neurochemicals, and other pharmacological agents can be applied easily (either in the bath or in the pipette), in defined concentrations, to both the external and internal surfaces of the membrane; (b) several chemicals or ions can be tested on one patch (or channel) either together or in sequence; and (c) several different drug or ion concentrations can be tested on the same membrane patch, thus facilitating generation of dose–response curves. In addition, the gating mechanism(s) behind the opening of channels can be pharmacologically tested more easily in a patch configuration.

The single-channel, cell-attached, and inside-out patch-clamp methods are particularly well-suited for the study of second messenger systems, and particularly for those systems (such as G-

protein-mediated events) that are "membrane-delimited" (see, e.g., ref. 25). Thus, in the cell-attached configuration, if a transmitter or other receptor regulator (first messenger) applied externally in the bathing solution—but not when applied from within the pipette—alters single-channel function (e.g., opening or closing of the channel recorded), the receptor must be remote from the recorded channel and mediation of the event by a diffusible second messenger is suspected. If the transmitter or regulator alters channel function even when applied via the recording pipette to an inside-out patch (where a soluble second messenger would diffuse away from the system), a membrane-delimited system is a likely candidate for an elegant example of this approach in opiate responses of locus coeruleus neurons.

A major disadvantage of the single-channel patch-clamp method is the necessity to use cultured or acutely isolated cells; such models seem to allow formation of better gigohm seals, probably because the relative lack of overlying glia or other supporting cells facilitates close apposition and seal of the pipette to the neuronal membrane. In addition, compared to cells lying within a slice preparation, the thin or nonexistent level of tissue and/or fluid overlying the recorded neuron reduces the capacitance in the recording system and allows better recording characteristics for the small-current signals generated by single channels. Therefore, most single-channel studies to date have been limited to cultured or isolated neurons. However, new refinements have allowed whole-cell patch (and some single-channel) recording in brain slices, and even in vivo (see below).

In part because of the free exchange of contents between the cell and the pipette, the whole-cell method has several additional advantages and disadvantages. First, unlike the single-channel methods, the whole-cell method can be used routinely in brain-slice preparations as well as in vivo. Also, with this method one can adjust the ionic balance and contents of the cell merely by placing the appropriate buffers and salts in the pipette solution. Furthermore, second messengers and drugs affecting them can be placed directly in the pipette solution for diffusion into the cell. For selective blockade of certain channel types (e.g., K+-selective channels), toxins (e.g., tetraethylammonium) or appropriate ions (e.g., Cs+) can also be placed in the pipette. And finally, dyes such as lucifer yellow or biocytin can be placed in the pipettes for quicker and more complete filling of the neurons than with the standard intracellular "sharp" pipettes.

The major disadvantages of the whole-cell method usually stem from the same properties that confer the advantages. Thus, because it is usually not possible to know the exact ionic or second

messenger composition of the normal resting cell, there is always the risk that essential cell constituents (e.g., cyclic AMP) will diffuse out of the cell into the pipette (which usually constitutes a much larger volume than that of the cell). It is thought that such diffusion accounts for the slow "run-down" in some cells of certain currents such as the L-type Ca2+ current. Attempting to replace such lost constituents (e.g., cyclic AMP, ATP, GTP) by adding them to the pipette solution can help alleviate some of these problems. The use of a nystatin "barrier" in the pipette tip, which allows passage of small monovalent ions (e.g., for current injection) but prevents the diffusion of most divalent and large nonionic constituents (e.g., proteinaceous buffers and components of phosphorylation systems) from the cell, is another procedure for reducing these problems.

As alluded to above, there are many advantages of the various imaging approaches, the most important being the ability to measure ionic or potential changes via relatively noninvasive methods. However, it should be remembered that most of these methods require dyes and/or light exposure of some sort that can distinctly alter cell function over time. In addition, most of these methods require relatively isolated or thin preparations, so that microscopic observation can be performed.

Relevance to Neuropsychopharmacology

Implicit in these studies of ion channels and their related receptors is the idea that a substantial molecular and electrophysiological understanding of the function of each structural element or subelement will allow the rational development of more effective and selective pharmacological agents and, ultimately, better therapeutic drugs. For example, understanding what part (amino acid residues, phosphorylated region, etc.) of the *N*-methyl-D-aspartate (NMDA) receptor-channel complex is affected by alcohol could lead to the development of a drug to blunt the alcohol antagonism of NMDA receptor function. Because the anti-NMDA effect of ethanol is thought to account for many aspects of alcohol intoxication, this drug could be the "silver bullet," sought by alcohol researchers for years, for rapidly reversing ethanolinduced intoxication. In addition, all of the single- channel methods have the capability of being applied to human samples (e.g., slices or cultures from biopsies), both from normal tissue and from diseased brains. The single-channel (and molecular) data from these two sources could then be compared for indications of the structural source of the abnormalities or malfunction; with this sort of

knowledge base, more rational drug development or intervention procedures would lead to new, more effective treatments for the disease.

Electroencephalography

Electroencephalography (**EEG**) is the recording of electrical activity along the scalp. EEG measures voltage fluctuations resulting from ionic current flows within the neurons of the brain.[2] In clinical contexts, EEG refers to the recording of the brain's spontaneous electrical activity over a short period of time, usually 20–40 minutes, as recorded from multiple electrodes placed on the scalp. Diagnostic applications generally focus on the spectral content of EEG, that is, the type of neural oscillations that can be observed in EEG signals. In neurology, the main diagnostic application of EEG is in the case of epilepsy, as epileptic activity can create clear abnormalities on a standard EEG study.[3] A secondary clinical use of EEG is in the diagnosis of coma, encephalopathies, and brain death. EEG used to be a first-line method for the diagnosis of tumors, stroke and other focal brain disorders, but this use has decreased with the advent of anatomical imaging techniques with high (<1 mm) spatial resolution such as MRI and CT. Despite limited spatial resolution, EEG continues to be a valuable tool for research and diagnosis, especially when millisecond-range temporal resolution (not possible with CT or MRI) is required. Derivatives of the EEG technique include evoked potentials (EP), which involves averaging the EEG activity time-locked to the presentation of a stimulus of some sort (visual, somatosensory, or auditory). Event-related potentials (ERPs) refer to averaged EEG responses that are time-locked to more complex processing of stimuli; this technique is used in cognitive science, cognitive psychology, and psychophysiological research.

Source of EEG activity

The brain's electrical charge is maintained by billions of neurons. Neurons are electrically charged (or "polarized") by membrane transport proteins that pump ions across their membranes. Neurons are constantly exchanging ions with the extracellular milieu, for example to maintain resting potential and to propagate action potentials. Ions of similar charge repel each other, and when many ions are pushed out of many neurons at the same time, they can push their neighbours, who push their neighbours, and so on, in a wave. This process is known as volume conduction. When the wave of ions reaches the electrodes on the scalp, they can push or pull electrons on the metal on the electrodes. Since metal conducts the push and pull of electrons

easily, the difference in push or pull voltages between any two electrodes can be measured by a voltmeter. Recording these voltages over time gives us the EEG.[4]

The electric potential generated by single neuron is far too small to be picked up by EEG or MEG.[5] EEG activity therefore always reflects the summation of the synchronous activity of thousands or millions of neurons that have similar spatial orientation. If the cells do not have similar spatial orientation, their ions do not line up and create waves to be detected. Pyramidal neurons of the cortex are thought to produce the most EEG signal because they are well-aligned and fire together. Because voltage fields fall off with the square of distance, activity from deep sources is more difficult to detect than currents near the skull.[6]

Scalp EEG activity shows oscillations at a variety of frequencies. Several of these oscillations have characteristic frequency ranges, spatial distributions and are associated with different states of brain functioning (e.g., waking and the various sleep stages). These oscillations represent synchronized activity over a network of neurons. The neuronal networks underlying some of these oscillations are understood (e.g., the thalamocortical resonance underlying sleep spindles), while many others are not (e.g., the system that generates the posterior basic rhythm). Research that measures both EEG and neuron spiking finds the relationship between the two is complex with the power of surface EEG in only two bands (gamma and delta) relating to neuron spike activity.[7]

Clinical use

A routine clinical EEG recording typically lasts 20–30 minutes (plus preparation time) and usually involves recording from scalp electrodes. Routine EEG is typically used in the following clinical circumstances:

- to distinguish epileptic seizures from other types of spells, such as psychogenic non-epileptic seizures, syncope (fainting), sub-cortical movement disorders and migraine variants.
- to differentiate "organic" encephalopathy or delirium from primary psychiatric syndromes such as catatonia
- to serve as an adjunct test of brain death
- to prognosticate, in certain instances, in patients with coma
- to determine whether to wean anti-epileptic medications

At times, a routine EEG is not sufficient, particularly when it is necessary to record a patient while he/she is having a seizure. In this case, the patient may be admitted to the hospital for days or even weeks, while EEG is constantly being recorded (along with time-synchronized video and audio recording). A recording of an actual seizure (i.e., an ictal recording, rather than an inter-ictal recording of a possibly epileptic patient at some period between seizures) can give significantly better information about whether or not a spell is an epileptic seizure and the focus in the brain from which the seizure activity emanates.

Epilepsy monitoring is typically done:

- to distinguish epileptic seizures from other types of spells, such as psychogenic non-epileptic seizures, syncope (fainting), sub-cortical movement disorders and migraine variants.
- to characterize seizures for the purposes of treatment
- to localize the region of brain from which a seizure originates for work-up of possible seizure surgery

Additionally, EEG may be used to monitor certain procedures:

- to monitor the depth of anesthesia
- as an indirect indicator of cerebral perfusion in carotid endarterectomy
- to monitor amobarbital effect during the Wada test

EEG can also be used in intensive care units for brain function monitoring:

- to monitor for non-convulsive seizures/non-convulsive status epilepticus
- to monitor the effect of sedative/anesthesia in patients in medically induced coma (for treatment of refractory seizures or increased intracranial pressure)
- to monitor for secondary brain damage in conditions such as subarachnoid hemorrhage (currently a research method)

If a patient with epilepsy is being considered for resective surgery, it is often necessary to localize the focus (source) of the epileptic brain activity with a resolution greater than what is provided by scalp EEG. This is because the cerebrospinal fluid, skull and scalp *smear* the electrical potentials recorded by scalp EEG. In these cases, neurosurgeons typically implant strips and grids of electrodes (or penetrating depth electrodes) under the dura mater, through either a craniotomy or a burr hole. The recording of these signals is referred to as electrocorticography (ECoG), subdural EEG (sdEEG) or intracranial EEG (icEEG)--all terms for

the same thing. The signal recorded from ECoG is on a different scale of activity than the brain activity recorded from scalp EEG. Low voltage, high frequency components that cannot be seen easily (or at all) in scalp EEG can be seen clearly in ECoG. Further, smaller electrodes (which cover a smaller parcel of brain surface) allow even lower voltage, faster components of brain activity to be seen. Some clinical sites record from penetrating microelectrodes.[2]

History

A timeline of the history of EEG is given by Swartz.[54] Richard Caton (1842–1926), a physician practicing in Liverpool, presented his findings about electrical phenomena of the exposed cerebral hemispheres of rabbits and monkeys in the British Medical Journal in 1875. In 1890, Polish physiologist Adolf Beck published an investigation of spontaneous electrical activity of the brain of rabbits and dogs that included rhythmic oscillations altered by light.

In 1912, Russian physiologist, Vladimir Vladimirovich Pravdich-Neminsky published the first animal EEG and the evoked potential of the mammalian (dog).[55] In 1914, Napoleon Cybulski and Jelenska-Macieszyna photographed EEG-recordings of experimentally induced seizures.

German physiologist and psychiatrist Hans Berger (1873–1941) recorded the first human EEG in 1924.[56] Expanding on work previously conducted on animals by Richard Caton and others, Berger also invented the electroencephalogram (giving the device its name), an invention described "as one of the most surprising, remarkable, and momentous developments in the

history of clinical neurology".[57] His discoveries were first confirmed by British scientists Edgar Douglas Adrian and B. H. C. Matthews in 1934 and developed by them.

In 1934, Fisher and Lowenback first demonstrated epileptiform spikes. In 1935 Gibbs, Davis and Lennox described interictal spike waves and the 3 cycles/s pattern of clinical absence seizures, which began the field of clinical electroencephalography. Subsequently, in 1936 Gibbs and Jasper reported the interictal spike as the focal signature of epilepsy. The same year, the first EEG laboratory opened at Massachusetts General Hospital.

Franklin Offner (1911–1999), professor of biophysics at Northwestern University developed a prototype of the EEG that incorporated a piezoelectric inkwriter called a Crystograph (the whole device was typically known as the Offner Dynograph).

In 1947, The American EEG Society was founded and the first International EEG congress was held. In 1953 Aserinsky and Kleitman describe REM sleep.

In the 1950s, William Grey Walter developed an adjunct to EEG called EEG topography, which allowed for the mapping of electrical activity across the surface of the brain. This enjoyed a brief period of popularity in the 1980s and seemed especially promising for psychiatry. It was never accepted by neurologists and remains primarily a research tool.

Various uses

The EEG has been used for many purposes besides the conventional uses of clinical diagnosis and conventional cognitive neuroscience. Long-term EEG recordings in epilepsy patients are used for seizure prediction. Neurofeedback remains an important extension, and in its most advanced form is also attempted as the basis of brain computer interfaces. The EEG is also used quite extensively in the field of neuromarketing. There are many commercial products substantially based on the EEG.[*citation needed*]

Honda is attempting to develop a system to enable an operator to control its Asimo robot using EEG, a technology it eventually hopes to incorporate into its automobiles.[58]

EEGs have been used as evidence in trials in the Indian state of Maharastra.[59][60]