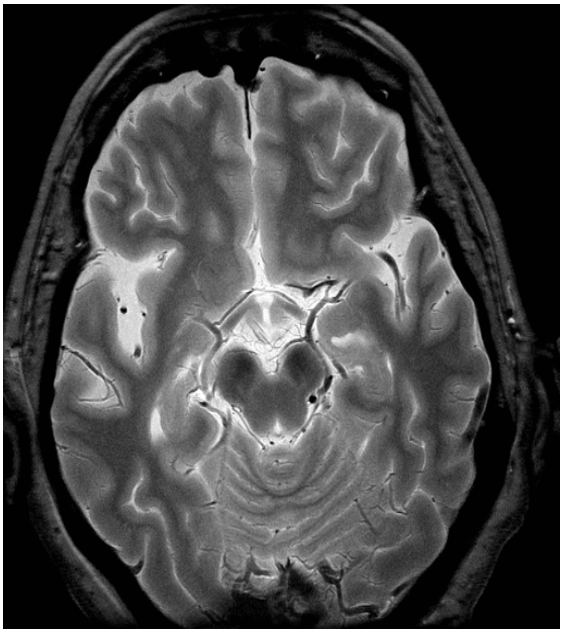
The Active Brain.

This chapter is intended to serve as an introduction to the basic principles of neurophysiology that are especially relevant to the neuroimager. After a brief historical overview we consider in detail the nature of neural signaling at the cellular level and explore the general concepts of regional specialization within the brain and the manner in which functional neuroimaging might be used to gain insight into the physiology of perception and cognition.

Introduction.

Consider this MRI image below. Today's scanners are capable of making images of the human body with amazing clarity. By methods we will discuss in more detail later in this book, the image was acquired non-invasively from outside the body, and the subject was exposed to no harmful radiation and no known or likely safety hazard. The *resolution* in this picture (the smallest details that can be distinguished) is just a few tenths of a millimeter – almost small enough to see single cells; the contrast between tissues ranges over an order of magnitude or more. The picture begs us to interpret it as a photograph, although it is more accurately viewed as a two-dimensional graph of tissue magnetic properties. MRI is popular, of course, as a diagnostic imaging tool in service to the radiologist to localize and characterize disease. In fact, a good neuroradiologist would quickly read this as a "negative" scan, meaning that there is no sign of pathology. What could be more perfect about this image? The simple fact that the subject was not alive at the time of this scan. For all of its glory, MRI generally is a *structural* imaging modality. It lacks dynamic access to tissue behaviors. The same is true of traditional X-Ray and X-Ray Computed Tomography (CT). To become a functional scan, something about ongoing activity in the tissues must be made visible.

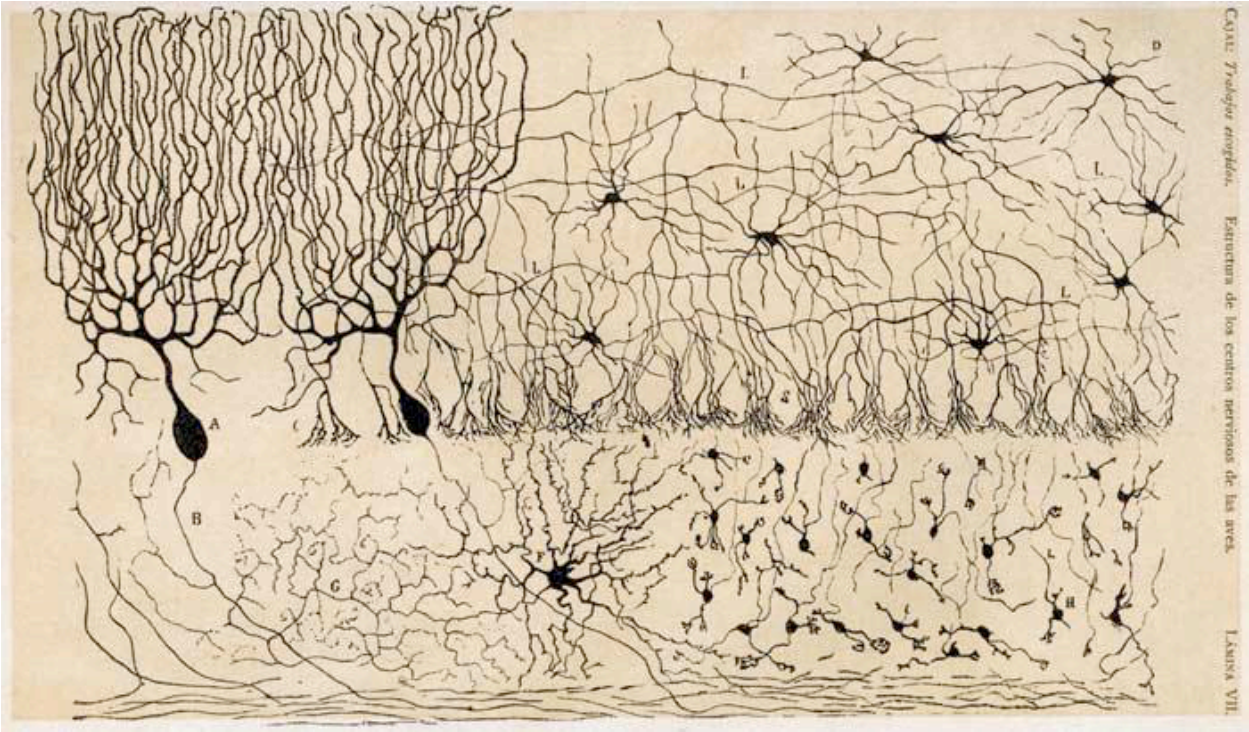


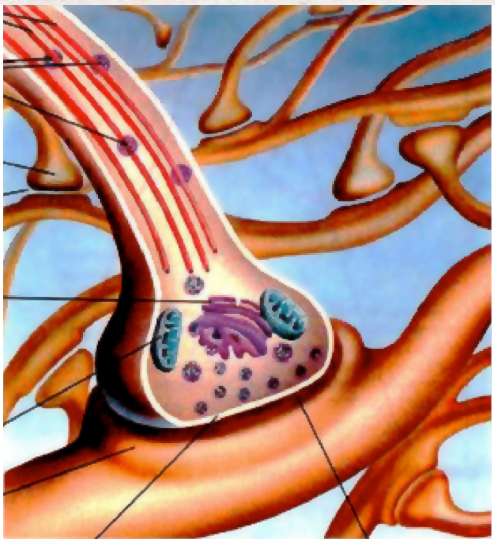
Functional MRI, like PET when used for functional neuroimaging, takes advantage of the energetic cost of neural information processing. As neurons communicate with one another, their signaling must be supported by metabolic activity that requires nutrients and creates waste, that moves ions and signaling molecules, that changes the sizes and physical properties of cells. Remarkably, to varying degrees each of these aspects of signaling can be made visible to MRI. While the bulk of this volume concentrates on blood oxygen based methods, in this chapter we will consider the means by which neurons communicate information and in so doing provide multiple windows to observe brain activity.

The Cytoarchitecture of the Brain.

The microstructure of the brain, and of other components of the nervous system, is decidedly complex. While to greater or lesser degrees the cells that form most body tissue are roughly spherical or ovoid, the cells of the brain – chiefly neurons and glial cells – have distinctly complex morphologies. The cell theory, that all organisms are composed of individual cells, was proposed by Thomas Schleiden, Theodor Schwann and Rodolf Virchow in the late 1830's and was revolutionary in understanding biology. Although the microscope had been invented centuries earlier, it was the advent of selective stains – especially the silver chromate stain of Camillo Golgi (1843-1926) – that first allowed anatomists to see the multitude shapes of neurons in detail. In examining stained neural tissue under the microscope, Golgi concluded that neural tissue was quite special and different, in that it was constituted of a syncytium of cells whose membranes were fused, such that their cellular contents (cytoplasm) was continuous, though the separate nuclei were retained. The Golgi method was exploited by Santiago Ramon y Cajal (who shared the 1906 Nobel prize in Medicine with Golgi) to systematically study the cytoarchitecture (the arrangement of cells in a tissue) of the neurons in the brain. Cajal came to an entirely different conclusion from Golgi, arguing instead that individual neurons were clearly identifiable and had distinct boundaries. This became known as the *neuron doctrine*. While Cajal's arguments were careful and logical, the debate remained active for many years. Conclusive proof of the neuron doctrine came with the advent of the electron microscope, however, which demonstrated clear cell membranes surrounding each neuron.

Neurons are immensely variable in their size, their shape and their cytoplasmic contents. Yet, they share some common features. Figure 2.3 shows several neurons (based on Golgi's original drawings) and highlights in addition to the cell body and nucleus common to all cells, the many *dendrites* which are filamentous processes that extend from the cell body and the single thin *axon* that may extend several hundred centimeters from the cell body. The magnified images in Figure 2.3A and B show the *synaptic boutons* and *axon hillock* that also are features distinctive to neurons.





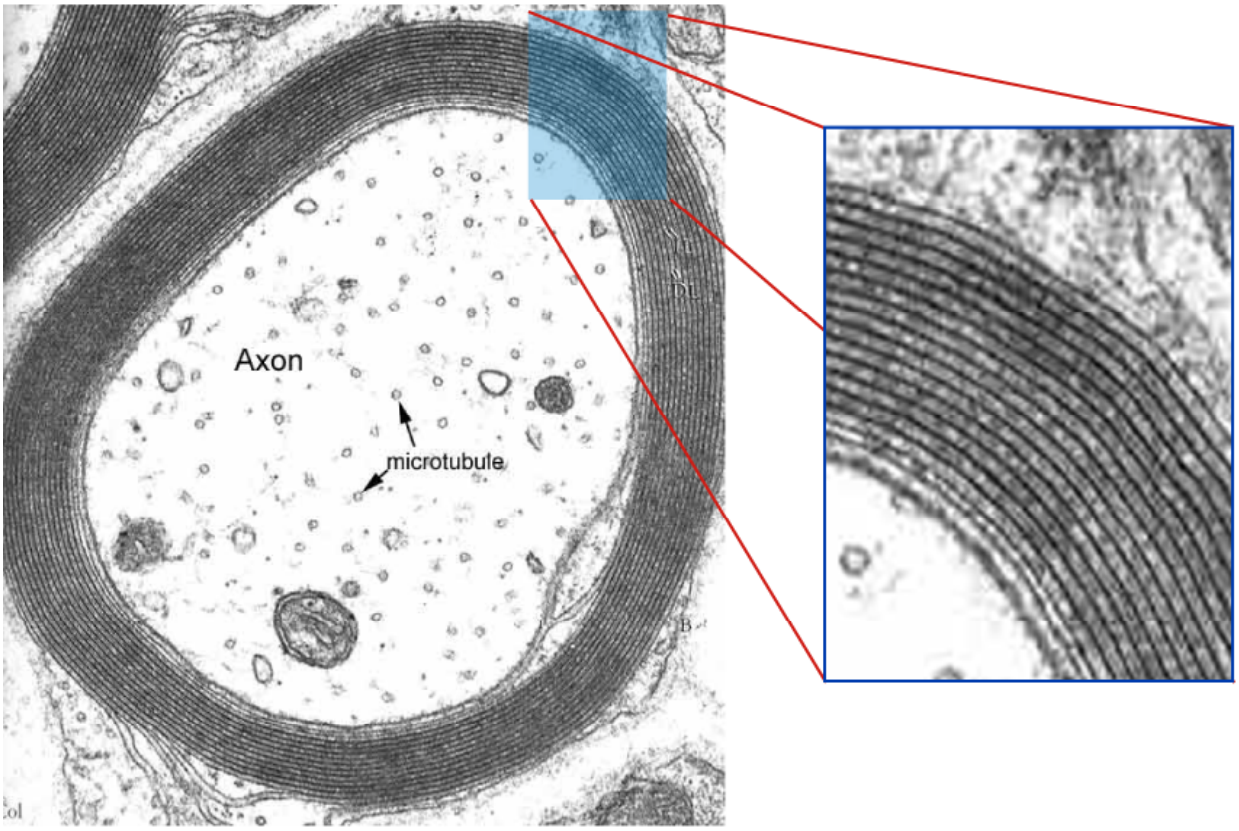
[Figure 2.3 here – neuron sketches]

In recent years, numerous means have identified molecular constituents unique to neurons as well. Of particular interest to the field of magnetic resonance is the presence of N-Acetyl-Aspartate (NAA), a molecule identified in 1956 as existing solely or primarily in neurons (Tallan, Moore et al. 1956). Because of its relatively high abundance, NAA may be seen readily by magnetic resonance spectroscopy techniques (see chapter XXX).

Neurons however, are neither the only, nor even the majority, of cells in the brain. Another group of cells unique to the nervous system are the glia. While early thinking held that the role of the glia was largely to provide a physical support matrix for neurons, their functions have been discovered to be very diverse, including several roles

in information signaling. Some clue to this can be seen by viewing the microstructure of the brain and peripheral nervous system. Two types of glia, the oligodendrocyte (in the central nervous system) and the Schwann cell (in the peripheral nervous system) form a layered wrapping about the axons of neurons.

Like all animal cells, the membrane of the glia is made up of a bilayer of lipid – fatty, water insoluble, non-polar molecules – that provide a barrier to electrically charged particles. When wrapped around the neurons, this membrane is called *myelin*. Myelin provides a substantial electrical boundary, as well as a limitation to the motion of water, which has an uneven charge distribution. The electrical properties of myelin are important in signaling and its disruption in disease (e.g., multiple sclerosis) is clinically important. The barrier that myelin presents to water motion causes the diffusion of water in the brain to be *anisotropic* such that water diffuses preferentially along axons rather than across the them. This property can be made visible by magnetic resonance ((Stejskal and Tanner 1965), and chapter XXX) and is the basis of *diffusion imaging*. Finally, the magnetic resonance signal from fat and water differs in several ways, and the myelin is one of the determinants of the contrast in MRI. For example, in figure 2.1, the cortex, or outer layers, of the brain, appear lighter then the myelin, which makes up a large fraction of the brain tissue and, in that figure, appears in dark gray.



The glial cells have many known functions in the brain that go well beyond their role in architectural scaffolding. Relevant to our subject among these are the metabolic processing of substances used in neural signaling, provision of energy to the neurons, control and regulation of blood flow and facilitation of electrical signals. They may also have more direct roles in chemical and electrical signaling in the brain. We will see that

the glial role in metabolism may be an important factor in the signals used in functional neuroimaging.

Functional Specialization of the Brain.

The majority of the current work in functional neuroimaging addresses the question of regional localization of brain function. This approach to an understanding has not always been dominant, though it is much in vogue today.

At the gross level the early anatomists viewed the brain as a largely amorphous substance, a point of view that must surely have been emphasized by the gelatinous character of the brain when removed from the skull. The contents of the cerebrum at this level of detail would appear to be a rich blood supply, a clear fluid (which we recognize now is cerebrospinal fluid, or CSF) and the jelly-like brain. As the heart had long been understood to be the seat of the soul, the brain was seen primarily as a biological device for cooling the blood.

That the brain has a function in conscious information processing is a fact established easily and long ago, simply by seeing the effects of injury to the head compared to other parts of the body. That the brain might be mechanistically involved, however, was much more difficult to see, and generally required the development of technologies such as electrical recording and microscopy to determine. Through the seventeenth century, the dominant theory was similar to that espoused by René Descartes (1596-1650), that the brain (more specifically, the pineal gland) was the site at which God interacted with the physical body of the human, and that consciousness was quite separable from the biological form. The general understanding was that the nerves communicated with muscles through some sort of fluid, as it was clear already that severing the nerve resulted in a loss of muscular control and the severing the connection from the eyes (the optic nerve) caused blindness.

The Cartesian view of the brain was challenged by the increasing skills and inquiries of the early neuroanatomists. It became clear, for example, that there was a regular organization to the folds of the brain surface and a highly regular anatomy to the connections of the nerves to regions within that brain, and thereby less tenable that the brain served some sort of simple cooling function. Franz Gall (1758-1828) was an Austrian physician and neuroanatomist living in Paris, who established, for example, the origin of the cranial nerves (that support vision, hearing, smell, etc…) was not in the cerebrum (the convoluted outer portion of the brain), but in the *medulla oblongata*, a bulbous structure in the core of the brain at the upper end of the spinal cord. Gall rather famously suggested that certain personality traits and mental disorders were physiologically based, rather than God-given, a view that is accepted today, but was clearly antithetical to the dominant religious tenets of his time. Reasoning that some of these traits might be localized to various parts of the brain, and that hypertrophy, or atrophy of these regions might affect the shape of the skull, Gall set about to study the relationship of the skull surface to personality types and mental illness. He developed a series of measurement schemes and instruments that became the devices used for *phrenology*. Phrenology developed into a very popular method (See Figure 2.5, for example) and supported many beliefs that we see in retrospect as racist in the extreme. Gall's charts were instantiated into porcelain models showing the association between

bumps on the skull and behaviors – such models sit on the desks of many neurologists and neuroscientists today, where we enjoy them for their quaint character and phrenology as a science is ridiculed. Nevertheless, he gave great momentum to the study of the relationship between brain structure and function that is the object of study for most practitioners of functional neuroimaging. Franz Gall, and his followers, including Jean Baptiste Bouillaud (1796-1881) and others, argued on the basis of brain lesions that language must be largely a function of the left half of the brain.

[Figure 2.5 – Psychograph here]

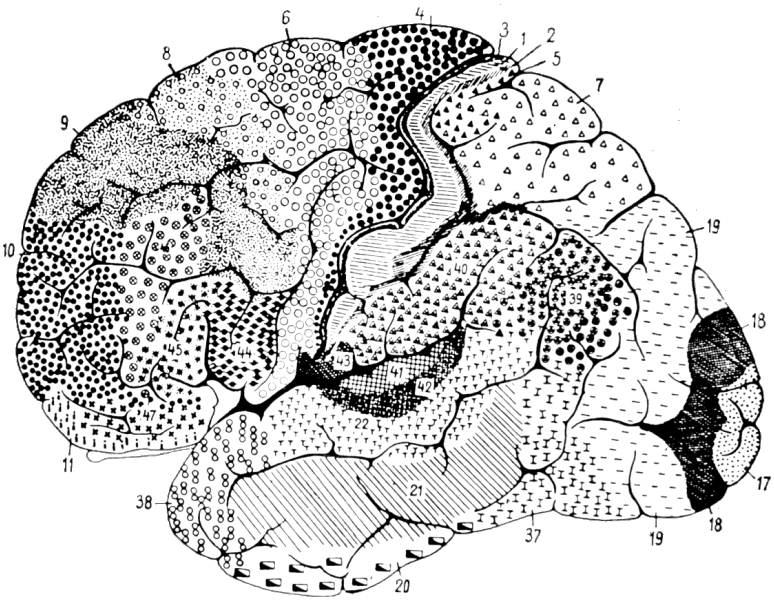
The controversy between those holding a localizationist view of brain organization, and those who believed that brain tissue was more pluripotent and holistic carried on through the 19th century. In a famous, and important, debate at the Société d'Anthropologie" in Paris, Ernest Aubertin (1825-1893), a student of Gall and Pierre Gratiolet (1815-1865) argued about the possibility of a language center in the frontal lobe of the brain, with Gratiolet suggesting that language could be sited anywhere within the brain. At that meeting, Aubertin declared that he would publicly renounce the concept of cerebral localization if anyone could show a single clinical case in which the loss of the faculty of articulate language could be shown without a corresponding lesion in the frontal lobes.

In the audience was Pierre-Paul Broca (1824-1880). Broca has been considered a child prodigy, entering his medical training at age 17 and graduating at age 21, with degrees in mathematics, physics and literature. Early in his career, he studied the microstructure (histology) of cartilage and bone, cancer pathology, the treatment of aneurysms and infant mortality. Broca was intrigued by the challenge that Aubertin set out and, in 1861 came across a patient who seemed to offer the opportunity to test Aubertin's theory. Later known as "tan", because this was the only syllable that the patient could pronounce, this patient was brought by Broca to Aubertin to confirm that, indeed, he had lost articulatory speech. Tan died only days after meeting Broca, who subsequently prepared a careful brain autopsy. Broca announced thereafter that the site of articulatory language was located precisely in the third frontal convolution of the left hemisphere of the brain, as predicted by Aubertin. This region of was named, "Broca's Area" and is referred to as such today.

By today's standards, the data Broca provided were not strong. Tan's brain was preserved and modern neuroimaging studies have shown that the lesion was huge and extended well beyond the third convolution (Signoret, Castaigne et al. 1984). Further, finding a single patient whose brain lesion corresponded with Aubertin's prediction did nothing to prove it and certainly did not address his challenge to be disproven by a lesion located elsewhere. Nevertheless, at that time, the pendulum in neuropsychological thinking swung far to the favor of localizationist thinking.

The functional architecture of the brain shares a remarkable relationship to its cellular microstructure. When the brain is sliced in cross section, the thickness of its cortex can be seen to very from about 1 to 4 mm. Under even modest magnification, the cortex itself appears to be composed of multiple lamina. Simple stains such as Nissl make this laminar structure clear. Early neuroanatomists were struck by both this layered organization and the conspicuous variation in the thickness of the layers from point to point in the brain. In a pioneering, and laborious, study Korbinian Brodmann (1868-

1918) systematically examined the association between the layering patterns and the gross location in cortex, across nine different+++ species. His 1919 atlas of the human brain divides the cortex into some 44 regions on the basis of the relative thickness of the cell layers (he identified 52 different areas in his cross-species comparisons, but 8 do not appear in his human atlas). These so-called Brodmann regions present a surface topography that is somewhat more regular than the sulci (fissures) and gyri (convex surfaces) of the folded brain, but in many cases are regularly aligned with the folding patterns. With the advent of electrophysiology, discussed below, it became clear that the Brodmann regions corresponded to functionally specialized brain regions, with a distinct cytoarchitectonic pattern corresponding, for example, to the primary sensory or motor regions.



[Figure 2.6 "Brodmann's Regions" Here]

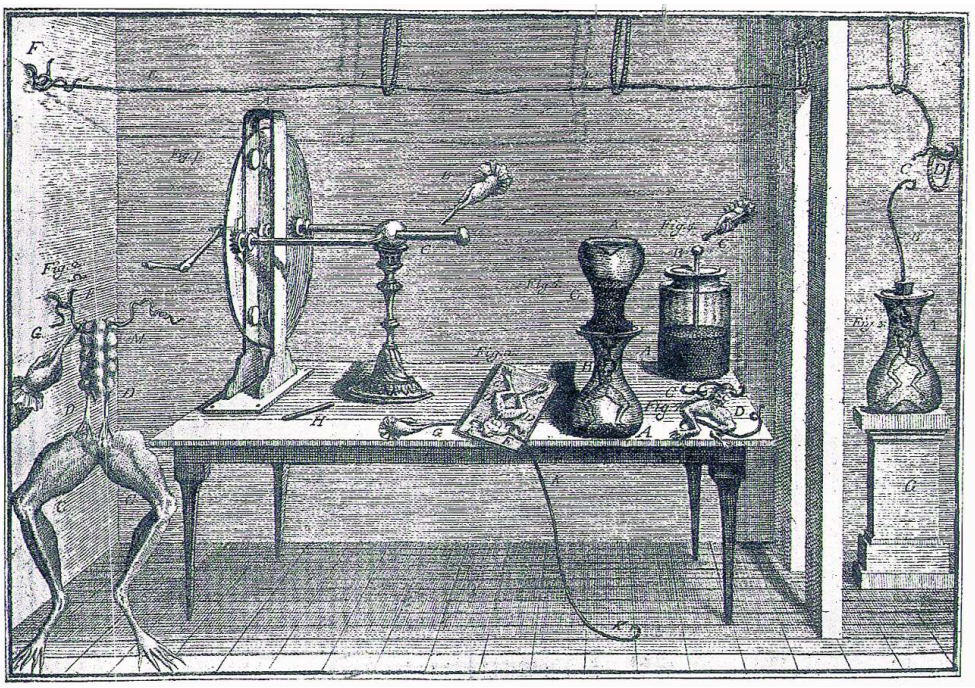
Functional specialization by brain region seems to be a core organizing principle of the human brain. Although the field of neuroscience is not generally so naïve as to believe that each cognitive, behavioral, motor or sensory function of the brain is associated with a single well-characterized brain structure, there have nevertheless emerged patterns of organization that are repeatable to varying degrees across individuals and across species. It seems clear that the organization of phylogenetically older features, such as muscular control and primary vision is more highly conserved than are the more abstract features of cognition such as mathematics, reasoning, or other learned skills. Although this is not intended as a comprehensive text in functional neuroanatomy, we will consider more of the functional topography of the brain later in this section.

The Electric Animal.

In the middle of the eighteenth century Luigi Galvani (1737-1798), an anatomist and experimentalist, was dissecting a frog and was surprised to find that the muscles of the animals' legs twitched when his assistant touched when the relevant nerve with a scalpel while electrical apparatus (an electrostatic generator or a Leyden jar) was operated nearby,

> "I had dissected and prepared a frog in the usual way and while I was attending to something else I laid it on a table on which stood an electrical machine at some

distance from its conductor and separated from it by a considerable space. Now when one of the persons present touched accidentally and lightly the inner crural nerves of the frog with the point of a scalpel, all the muscles of the legs seemed to contract again and again as if they were affected by powerful cramps."



[Figure 2.6 – Galvani lab here]

He then went on to observe and study the effects of touching the nerve to various metals and to other parts of the body of the animal. He understood that the force involved was electrical. Living, as he did, in a time when vitalism was a prevailing philosophy, Galvani reasonably concluded that the electricity was in fact the vital force itself and was generated by the body of the living animal; he can be excused for believing that this "animal electricity" was somehow distinct from the "artificial electricity" generated by friction (now known as static electricity) or the "natural electricity" of lightning.

Galvani's contemporary and friend, Alessandro Volta (1745-1827) had been studying the generation of electrical potential differences through the use of various combinations of metals and salts (a principle we will visit in more detail later, as we discuss the generation of the intracellular potential). Volta replicated Galvani's experiments, but came to a different conclusion, namely that the source of the electricity that caused the frog's leg to move was not the frog, but the metals in the scalpel used by Galvani's assistant. An impassioned, but friendly, debate followed. Of Galvani's work, Volta wrote that it, "contained one of the most beautiful and most surprising discoveries". As it happens, both Volta and Galvani were proven correct: Galvani had actually stimulated the nerves of the frog leg with the electrical potential generated by his instruments, but was able to do so because the natural means by which nerves communicate to muscles is itself electrical. Together the work of Galvani and Volta established that the basis of neural conduction was almost certainly electrical. In recognition of Galvani's work,

Volta termed his device for the measurement of electrical potential energy the galvanometer. Volta's work was similarly honored by the community at large, who use galvanometers to measure this energy in units of Volts, or voltage.

With the intense interest, at the time, in the study of things electrical, and with Galvani's important discovery, people worked enthusiastically in this area. Of special relevance was the work of the early neurophysiologists, Charles Roy and Charles Sherrington. Operating on the exposed brain of a dog, they noted that electrical stimulation of certain regions of the outer surface (cortex) of the brain resulted in movement of the anesthetized animal's leg. These experiments were significant for many reasons: for example, they indicated a relationship between brain electricity and muscle control and they added evidence that the brain was regionally specialized (a fact established previously by Paul Broca – see below). In their writings, these scientists note that immediately under the electrode stimulating site, the animal's brain became more pink, and they speculated that this was evidence of a local blood flow regulation, within the brain, that was associated with the brain metabolic activity (Roy and Sherrington 1890). Sherrington went on to share the 1932 Nobel prize in Physiology or Medicine with Edgar Adrian for their work studying the function of neurons. Working simultaneously, from a wholly different perspective, the noted psychologist William James observed that,

> "We must suppose a very delicate adjustment whereby the circulation follows the needs of the cerebral activity. Blood very likely may rush to each region of the cortex according as it is most active, but of this we know nothing." (James 1890)

That such a coupling between neural activity and blood flow exists is now a firmly established fact, although the details of the mechanism are remain an area of active investigation and significant controversy. For the time being, we will accept the observable phenomenon of a local increase in brain blood flow with brain activity, and leave a more detailed consideration for later. What is important here is that although brain activity is difficult to observe directly, blood flow is accessible by a variety of non-invasive means, including by magnetic resonance, and that these vascular effects can serve as a reasonable proxy for neural events.

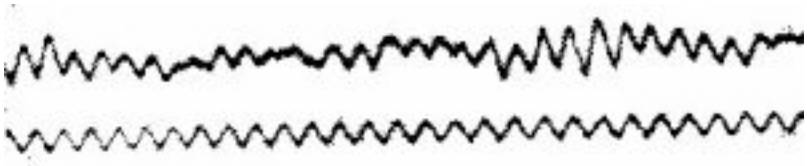
A parallel series of experiments recording the electrical potentials on the brain and scalp also emphasizes the importance of electrical currents in brain activity. In 1875, Richard Caton (1824-1926) reported on his experiments using a galvanometer to measure the potentials across and through the cortex. In his words:

> "In every brain hitherto examined, the galvanometer has indicated the existence of electric currents. The external surface of the grey matter is usually positive in relation to the surface of a section through it. Feeble currents of varying direction pass through the multiplier when the electrodes are placed on two points of the external surface of the skull. The electric currents of the grey matter appear to have a relation to its functions. When any part of the grey matter is in a state of functional activity, its electric current usually exhibits negative variation. For example, on the areas shown by Dr. Ferrier to be related to rotation of the head and to mastication, negative variation of the current was observed to occur

> whenever those two acts respectively were performed. Impressions through the senses were found to influence the currents of certain areas, e.g., the currents of that part of the rabbit's brain which Dr. Ferrier has shown to be related to movements of the eyelids, were found to be markedly influenced by stimulation of the opposite retina by light." (Caton 1875)

Thus, Caton was the first to describe the electrical activity *evoked* by brain processes. It is significant also that he described the fluctuating currents measured between points on the cortex. Although these are now understood to be the basis of the electroencephalogram (EEG), these were not the object of Caton's interest.

Hans Berger (1873-1941) was a reclusive and somewhat eccentric psychiatrist. Among his many interests was the study of psychic phenomena and telepathy – a history that likely impeded his later publications. Berger began observing the variations in electrical potential across the scalp surface and is credited with the discovery of the human EEG or "brain waves." With his son as a research subject, Berger used a Siemens double coil galvanometer attached to a simple chart recorder and demonstrated rhythmic fluctuations in voltage of about 100 μV with an oscillation frequency of about 10 Hz (see figure 2.7) (Berger 1929). Subsequently, this 8-12 Hz has become recognized widely as the alpha rhythm, following Berger's own terminology. These were a singular and remarkable finding and to this day, the multiple oscillatory signals that appear in the EEG are of unknown function and unclear original. They are, however, a stereotype feature of the mammalian brain and are used heavily by neurologists in the characterization of abnormalities and of sleep, among other states. Parenthetically Siemens, at the time a small company developing measurement instruments, has since become a multi billion dollar multi-national with an enormous presence in medical instruments and in MRI in particular, though they are no longer as active in EEG recordings.



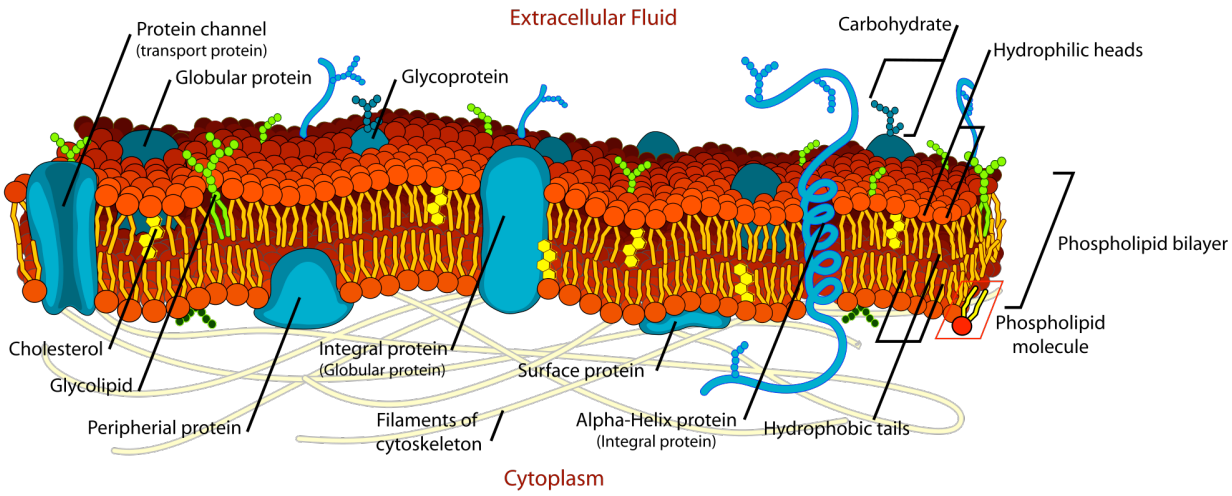
[Figure 2.7 Berger Alpha here]

In his studies Berger showed that the alpha rhythm tended to be a marker for resting state brain, while other rhythms of different frequency appeared when subjects concentrated on different tasks. For example, a higher frequency "beta" rhythm appeared when his subjects performed math problems. Berger carried out his experiments for five years in secrecy, and his public presence focused on telepathy, which he believed to be based on the propagation of these brain waves. Although he published his EEG findings in 1929, it took years for him to receive the credit due him. In 1937, more than a decade after his groundbreaking experiments, he was invited to speak, with tears in his eyes, before the Congress of Psychology in Paris, who was called the most distinguished of all the visitors – a phenomenal distinction, as he presided over the congress together with Nobelist Edgar Adrian. Even so, the historical linkage of the EEG to parapsychology might still color its perception in scientific circles today. We will

discuss more features of the EEG, and its integration into functional imaging, in later chapters.

Electrical Cells.

It is now known, as alluded to previously, that the membrane surrounding animal cells is composed of a bilayer of phospholipids. The phosphate containing head of these molecules is highly polar, meaning that there is an electrical charge separation. A similar separation of charge is found in water, such that relatively low energy states are created when water molecules align with the heads and form hydrogen bonds. Thus, the polar heads of the lipid membranes are *hydrophilic*. By contrast, the tails of the membrane lipids are made of chains of approximately hydrocarbon of about ten carbons in length. Because the electron affinity (electron negativity) of hydrogen and carbon is similar, in covalent bonds between these atoms there is a very even distribution of electrical charge from the shared electrons. As a consequence, the tails are *non*-polar and tend to repel water (they are *hydrophobic*).



[Figure 2.8 membrane lipid Here]

In a liquid suspension of membrane lipids in water, these molecules tend spontaneously to form a *bilayer* with the polar heads on the surfaces and the non-polar tails on the inside. The length of the membrane molecules gives the membrane a nominal thickness of about 5 nm (5×10^{-9} m). The principal structure of the membrane of eukaryotic cells is such a bilayer whose simple structure has important chemical properties. The cell membrane is highly impervious to water, because the highly polar water molecule cannot travel readily through the non-polar interior of the membrane. For similar reasons, the cell membrane resists the movement of charge molecules. The flow or movement of charge is known as electrical current (more specifically, the current is, i, is the first derivative of the charge, Q. $i = dQ/dt$) and the proportionality between the amount of electrical force applied (the Voltage) and the current is the electrical *resistance*, R, which is measured in units of Ohms. The latter relationship is known as

“Ohm’s Law”: V=*i*R. The cell membrane exhibits very high electrical resistance of several GigaOhms (>10^9 Ohms).

When a Voltage source is applied across an insulator, positive charge will build on one side of the insulator and negative charge on the other. The amount of charge is *inversely* proportional to the thickness of the insulating material. If the Voltage source is removed, the charge across the insulator will remain, held in place by the attractive force between the positive and negative charges. This property of insulators is called *capacitance* and is measured in units of Farads.

When charge is added to one side of an insulator, a matching charge will build up on the other side. For example, when positive charge is added to one side of a lipid bilayer, negative charge will build on the opposite side.

Therefore, a time-varying charge on one side will lead to an oppositely signed time-varying charge on the other, resulting in the flow of electrical current. The ratio between the amount of charge and the Voltage across an insulator is known as the *capacitance*, which is measured in Farads.

Berger, H. (1929). "Über das Elektroenzephalogramm des Menschen." Arch. Psychiatr. Nervenk. **87**: 527-570.

Caton, R. (1875). "The electric current of the brain." The British Medical Journal **2**: 278.

(complete article) After a brief resume of previous investigations, the author gave an account of his own experiments on the brains of the rabbit and the monkey. The following is a brief summary of the principal results. In every brain hitherto examined, the galvanometer has indicated the existence of electrical currents. The external surface of the grey matter is usually positive in relation to the surface of a section through it. Feeble currents of varying direction pass through the multiplier when the electrodes are placed on two points of the external surface, or one electrode on the grey matter, and one on the surface of the skull. The electric currents of the grey matter appear to have a relationship to its function. When any part of the grey matter is in a state of functional activity, its electric current usually exhibits negative variation. For example, on the areas shown by Dr. Ferrier to be related to rotation of the head, and to mastication, negative variation of the current was observed to occur whenever those two acts respectively were performed. Impressions through the senses were found to influence the currents of certain areas; e.g., the currents of that part of the rabbit's brain which Dr. Ferrier has shown to be related to movements of the eyelids, were found to be markedly influenced by stimulation of the opposite retina by light.

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> We must suppose a very delicate adjustment whereby the circulation follows the needs of the cerebral activity. Blood very likely may rush to each region of the cortex according as it is most active, but of this we know nothing.

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