7

Signals, Noise, and Thresholds

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7.1 Signals, Detection, and Measurement

Measurement is a quantitative observation and well known to be of great importance to science. However, measurements involving biological systems are complicated by the complexity of cells and tissues, particularly if fields are expected to interact weakly and field-induced changes are found to be small. Some key parameters, for example, temperature coefficient of a measured quantity, may be inadequately characterized, and related quantities may be determined incompletely (e.g., measurement or modeling of the time-dependent temperature throughout the volume of the biological system being studied). Detection is a special case of measurement, that is, the measurement is so coarse that an observer can only distinguish between "signal" and "no signal."

Generally speaking, the smaller the change in an observed quantity (e.g., cell biomass) due to a stimulus (e.g., an applied electromagnetic field), the more difficult the experimental interpretation. There may be multiple candidate causes if small changes in biomass are found, for example, any of many growth-altering biochemical changes, unnoticed and uncharacterized temperature variations, or even changes in ambient light or mechanical vibration. In physical science a model can often be made of the experiment. This allows estimates of the influence of various quantities and parameters on the expected experimental outcome (change in observed quantity in response to a stimulus) and is valuable in the interpretation of experiments. Similar approaches to bioelectromagnetics should also be valuable.

Consider an illustrative measurement on a biological system: a population of microorganisms contained within a glass toroid. Application of an alternating magnetic field to a primary coil wrapped around one part of the toroid induces an alternating current. The induced current can be measured with a coil wound around part of the toroid. The induced current is related to the electrical conductivity of the aqueous electrolyte. The electrical conductivity of the extracellular medium changes when small, charged metabolites are excreted, and measurement of microbial metabolic activity can thus be accomplished electrically. First observed in 1899 by a nulling technique [1], electrical impedance detection of microorganisms has received significant attention as a measurement method [2,3]. A toroidal device has actually been explored as the basis for determining microbial activity [4], with metabolic acid production causing a change in extracellular ion concentration (activity) and therefore creating a change in the electrical conductivity of the extracellular medium. But complications may arise. If cytotoxic chemicals leach from the glass, there can be a time-dependent poisoning of microbial activity. Ambient temperature changes couple through the glass to create internal temperature variations that alter the conductivity. In short, because electrical conductivity change has more than one candidate cause, this measurement system lacks specificity. This also illustrates a basic challenge to measurement of effects of electromagnetic fields on biological systems, namely, demonstrating both a statistically significant change and convincing evidence that it is the field interaction with the biological system, not an associated competing influence, that is responsible for the observation.

7.2 Specificity

Specificity is a hallmark of biological interactions involving biochemicals. A cell contains a large number of coexisting molecules whose interactions are not spontaneous but are instead highly regulated. Enzymes can be highly specific in the reactions they catalyze. Antibodies and receptor–ligand binding are also often specific. However, interactions of electromagnetic fields with a biological system are rather general. Magnetic fields interact indirectly by inducing electric fields and directly through magnetically sensitive reactions [5] and through interactions with magnetic material. Such magnetic materials may be contaminant ferromagnetic particles in the human body [6] or they may be biologically synthesized magnetite granules [7,8]. Electric fields interact nonspecifically with charge and polarizable material. Thus, unlike ligand–receptor biochemical interactions, there are no molecular receptors that are highly specific for electromagnetic fields. Instead, magnetic and electric sensory systems interact broadly and can be regarded as nonspecific. Evolved sensory systems are rather special. To date, it appears that biological, electric and magnetic field reception is indeed accomplished by organized systems.

Lack of electromagnetic field specificity has important implications for interpreting experiments. If an experiment quantifies a change in an observed parameter, the cause of the change is not automatically known. Continuing the example of cell growth determination based on biomass measurement, if an increase in biomass (or cell number) is associated with a field exposure, then additional analysis is needed to determine whether this change is due directly to the field or is instead due to interfering influences such as temperature change or biochemical concentration changes.

The challenge of specificity is not limited to weakly interacting fields. Consider the case of strong, electroporating fields *in vivo*, for which the motivation is local tumor treatment

or gene therapy. Strong fields can generate tissue movement by stimulating muscles and possibly also by bulk tissue polarization forces. Tissue motion can itself create membrane openings, and these can lead to biochemical transport [9–11]. Thus, observation of molecular uptake associated with electrical pulsing does not by itself show that electroporation is responsible. Specificity is an issue.

7.3 Signal-to-Noise Ratio

We adopt a recent discussion of the signal-to-noise ratio (S/N) for experiments with biological systems exposed to weakly interacting electromagnetic fields [12]. The observed quantity is x. For bioelectromagnetics experiments, examples of x include a local or spatially averaged transmembrane voltage change, temperature rise at a particular site, radioactivity of an incorporated unstable isotope, specific enzyme activity, intracellular calcium ion concentration, cell biomass, etc. Typically, experiments obtain data that can be characterized by their means and standard deviations, often presented as a bar chart. One bar of each bar pair represents the control result, and the other bar represents the exposed result. Each bar height represents the mean value, and the error bar is usually the standard deviation. (In some cases the error bars instead represent the standard error, that is, the uncertainty in the mean, rather than the standard deviation, but generally a report states which is being used.) Bar charts present a concise summary of an investigator's knowledge of the underlying natural distributions. The measured mean and standard deviation of the control distribution can be defined to be $\bar{x}_{con}$ and σ_{con}, respectively. Similarly, $\bar{x}_{exp}$ and σ_{exp} are the observed mean and standard deviation of the exposed distribution.

When repeating the same experiment and doing the same measurement many times over, one generally finds a Gaussian distribution of outcomes. This is because in a complex system there are many variables and sources of inaccuracy that are not under the control of the experimentalist. For the cumulative effect of all these imprecisions the central limit theorem becomes applicable. This theorem says that with many independent stochasticities involved, the outcome will be a Gaussian distribution [13]. As an example of this theorem in practice, do 100 coin tosses and record the number N of "heads." Repeat this experiment many times. The result will converge to a Gaussian distribution of N that is centered around 50.

The threshold for a field exposure effect occurs under conditions of detection, that is, the minimum change of x that is discernable using generally accepted statistical criteria. This is equivalent to determining whether or not the control statistical distribution and the exposed distribution are distinguishable (significantly different by accepted criteria). This requires sufficiently precise knowledge of the statistical distribution parameters. Increasing the number of determinations of the natural distribution generates more precise knowledge of its parameters. For example, if an investigator carries out a number, m_{con}, of determinations of x_{con} and another number, m_{exp}, of determinations of x_{exp}, then the empirically determined values can be reported as

$$x_{con} = \bar{x}_{con} \pm \frac{\sigma_{con}}{\sqrt{m_{con}}} \quad \text{and} \quad x_{exp} = \bar{x}_{exp} \pm \frac{\sigma_{exp}}{\sqrt{m_{exp}}} \tag{7.1}$$

The ratio $\sigma/\sqrt{m}$ actually represents the aforementioned standard error. Increasing the number of determinations reduces the standard error and the ensuing uncertainty in the mean. However, it does not decrease the standard deviation, σ_{exp}, of the underlying distribution, which is assumed unperturbed by the measurement process.

As the means $\bar{x}_{con}$ and $\bar{x}_{exp}$ become better known through more determinations, the potential distinguishability of the two distributions increases. The p-value of the experiment is often reported as a measure of this distinguishability. The p-value is the probability that the two means would be found to be as different as observed (or even more different) purely because of random variability. For example, $p = 0.01$ indicates that there is only a 1% chance that the difference between the control mean and the exposed mean would be due to the (assumed) random variability of the measured quantity, namely, the standard deviation [14]. After an investigator completes an experiment and finds a reasonably small p-value (.01 and .05 are widely used values), it is a common practice for the investigator to report that an effect due to the field exposure has occurred. However this assumes specificity, namely, that the field exposure rather than an associated competing influence is responsible. Indeed, a small p-value supports an effect of some sort but not necessarily one due to the field during the exposure. Additional analysis that considers other competing influences such as temperature variations, vibrations, and chemical concentration variations [15,16] is required for that conclusion.

Bioelectromagnetics experiments with weakly interacting fields typically involve determination of changes with respect to background values of, for instance, transmembrane voltage, fluorescence intensity, enzyme activity, or cell number. Observed changes in "exposed" relative to "control" are generally small. At the other extreme, strongly interacting fields create large changes with respect to background, for example, molecular uptake by electroporation (see Chapter 9 on electroporation in Ref. [127]). For the "weakly interacting" situation the uncertainties (error bars) are about the same for exposed and control. However, there is another figure of merit, distinct from the p-value, namely, an empirically determined signal-to-noise ratio $(S/N)_{obs}$, which is associated with the observation and which is presumed due to the underlying statistical distributions for the control and exposed cases. Classical detection theory shows that the associated distributions are expected to be Gaussians [17].

In continuation of a recent discussion [12], we consider the observed signal (S_{obs}) to be the difference between the control and the exposed means, and the observed noise (N_{obs}) as the standard deviation of the control distribution [17]. This yields

$$S_{obs} = \bar{x}_{exp} - \bar{x}_{con} \quad \text{and} \quad N_{obs} = \sigma_{con} \tag{7.2}$$

so that the empirically determined signal-to-noise ratio is the magnitude of

$$(S/N)_{obs} = \frac{\bar{x}_{exp} - \bar{x}_{con}}{\sigma_{con}} \tag{7.3}$$

Like the p-value $(S/N)_{obs}$ is a measure of the distinguishability of the two distributions. However, unlike the p-value, the signal-to-noise ratio is an inherent characteristic of the biological system, its environment, and a particular field exposure and does not depend on the number of determinations. In this view, S_{obs} is the observed change and is assumed to be a measure of the strength of the perturbation to the biological system by the field exposure. N_{obs} is a measure of the natural variability in the system for the conditions of the experiment. In the absence of an exposure, N_{obs} provides the appropriate scale to gauge the strength of S_{obs}.

$(S/N)_{obs}$ is based only on experimental determinations of x. However, in many cases the field exposure is believed to *indirectly* alter x. According to this general hypothesis, the field exposure affects one or more molecular-level biochemical processes through physical interactions. In this sense, the exposure is creating a "primary" molecular change, which is then amplified through a biochemical cascade that creates a downstream change. It is this downstream change that is eventually measured. The signal-to-noise ratio cannot be increased by the amplification process. Later in this chapter, we will describe how amplification generally adds noise to a signal.

7.4 Detection Criteria

The criterion $(S/N) \leq 0.1$ is a very conservative basis for ruling out a particular class of biophysical mechanism for a given field exposure. Similarly, the criterion $(S/N) \geq 10$ is a conservative basis for ruling in a candidate biophysical mechanism for a given exposure, retaining that biophysical mechanism hypothesis for further evaluation. This approach provides a quantitative basis for rejecting or accepting hypothetical biophysical mechanisms as candidate explanations for an experimental measurement. The traditional choice $(S/N) \approx 1$ is a useful but somewhat arbitrary dividing line, which indicates conditions for which an effect might appear. $(S/N) \leq 0.1$ and $(S/N) \geq 10$ provide criteria for stronger conclusions, allowing rejection or provisional retention of a biophysical mechanism hypothesis.

We should recognize that thresholds are defined by generally accepted statistical criteria. The widely used p-values of .01 and .05 are examples of such generally accepted statistical values. In the case of signal-to-noise ratios, a commonly accepted value is $(S/N) \approx 1$, where the approximately equal symbol denotes the imprecision. Specifically, if (S/N) (empirical or theoretical) exceeds 1, then the threshold is viewed as being exceeded. Similary, if (S/N) is less than 1, the response is interpreted as subthreshold. Clearly, it makes little sense to take a strong position if (S/N) is close to 1. But, as noted above, if the signal-to-noise ratio is significantly greater or less than 1, then some confidence can be attached to the result. In short, a threshold is imprecise but nevertheless a useful guide.

7.5 Equilibrium Noise

In this section we will examine how Brownian noise, the simple random motion of molecules due to thermal agitation, interferes with the coupling of an electromagnetic field to a biochemical system. Some organisms have evolved an ability to sense and effectively "measure" electric and magnetic fields. We will see that the thermal noise that a signal has to compete against sets fundamental limits on detectability. We will also see how evolution has come up with structures to optimize the signal-to-noise ratio in sensory perception.

Fish generally carry a small dipolar field relative to the water that they swim in. Sharks, skates, and rays have developed special organs to detect such fields [18,19] and they use this ability to pinpoint the position of their prey when they get close and the water is too turbulent to rely on smell. To be effective, the shark should be able to sense its prey

instantaneously. So, for the signal not to be mistaken for Brownian noise and for Brownian noise not to be mistaken as a signal, a signal should carry an energy that is significantly larger than kT. kT constitutes the average energy in the thermal noise band [20]. This baseline criterion already works to explain some of the physiology of the electric sensing organs. The electric fields are picked up by the ampullae of Lorenzini. These ampullae terminate at pores in the skin around the fish's head. They are enclosed in a highly resistive material and are filled with a very conductive gel. The eventual setup is equivalent to an electrical wire with no voltage drop inside. These ampullae are, furthermore, well insulated against electrical noise that originates from the fish's own physiology. Two pores that are about 10 cm apart on the surface of the fish's head can, on the inside ends, be separated only by a few nanometers. A field of 500 nV/m can be detected. Two pores that are 10 cm apart on the surface could thus transfer 50 nV into a transmembrane potential.

By having a lot of ion channels that are sensitive to such small voltage variations, the thermal noise can be effectively averaged out. With N ion channels instead of just one, N times as much signal strength is picked up. The thermal noise at each channel is independent of that at any other channel. The noise is zero-average and the noise variances are added up for N channels. So the average noise amplitude will be only $\sqrt{N}$ times as large if N channels are involved instead of one. After detection, the fish has to amplify this signal to the millivolt range that the nervous system operates with. Amplifier noise constitutes a problem that builders of electric circuits have dealt with for decades. Amplifier noise is nonequilibrium noise, and we will discuss it in the next section. Over the past decade, researchers have built up a good and detailed understanding of the physiology [21] and physics [22,23] of the fishes' amplification system.

Many animal species have the ability to detect the geomagnetic field. Two mechanisms have been proposed for magnetosensitivity. The first mechanism involves chemical transitions that are sensitive to external magnetic fields. Upon excitation by light, many polyatomic molecules will start transiting between the singlet ground state, the singlet excited states, and the excited triplet state. The energy difference between a singlet ($\uparrow\downarrow$) state and a triplet ($\uparrow\uparrow$) state is affected by an external magnetic field. This energy difference is generally small for fields of the magnitude of the Earth's magnetic field. But the magnetism that living cells generate is even smaller. A magnetically sensitive reaction of this type is therefore not subject to significant thermal noise. However, a detection limit can be established by considering a model in which reacting product molecules can bind to receptors. There is an innate stochasticity in chemical reactions; rates represent an average behavior, and there is a Gaussian distribution around this average. This is called fundamental chemical noise, and we will come back to it later in this chapter. In this model, the average number of occupied receptors varies with the magnetic field, and the detection limits are set by this fundamental chemical noise [24]. The fact that many bird species actually need light for their magnetic compass to work is a strong indication that singlet–triplet transitions are involved in the navigation. Recently, additional evidence was found when it turned out that robins get disoriented when they are subjected to an RF magnetic field that oscillates at the singlet–triplet resonance frequency [5] (for more details, see Chapter 6 on free radical models).

The second mechanism that has been proposed to explain magnetosensitivity involves the small (<100 nm in diameter) granules of magnetite (Fe_3O_4). This material, also known as lodestone, is biochemically formed and has about 30% of the magnetic strength of pure Fe. In the 1970 s, it was discovered that certain microbes use single-domain magnetite granules, also called magnetosomes, as a kind of rudder to help them stay under water right at the interface between the water and the mud at the bottom. There is a force trying to align the magnetic granule(s) with the Earth's magnetic field,

and the microbe thus "finds out" what its own orientation is relative to the inclination of the Earth's magnetic field [25,26]. For a single-domain magnetite granule of about 100 nm in diameter, the product μB of the magnetic moment μ and the Earth's magnetic field B amounts to about $5kT$. This $5kT$ alignment is sufficient to exceed the kT thermal agitation in the granule's rotation. In higher animals it appears that the granules are commonly embedded in biopolymers and lined up to form a rigid linear rod. Such an alignment effectively increases the magnetic moment and thereby the sensitivity to small variations in the magnetic field [27]. Indications are that there can be up to a million magnetite-containing cells in the brain of almost any animal. Even humans, who exhibit no apparent magnetosensitivity, have magnetite in their brain tissue [7,8].

The intensity of the Earth's magnetic field varies from 25 to 65 μT, and the direction varies from parallel to perpendicular to the Earth's surface. The magnetic sensitivity of, for instance, homing pigeons has been shown to be such that field variations smaller than 10 nT can be detected. With such a sensitivity the pigeon can use the change of the magnetic field vector to furnish itself a kind of global positioning system (GPS) [29]. Recent data indicate that some birds incorporate both magnetite and singlet–triplet chemistry in their magnetosense [5].

It is tempting to hypothesize that extremely low-frequency (ELF) radiation or microwave radiation could have a physiological effect through the interactions with magnetosomes. Cells produce their own electricity and concurrent electric noise. But there is no significant endogenous magnetic field noise. So the magnetic part of ELF radiation or microwave radiation would not have to compete against such endogenous biological noise. The average 24-h personal 60-Hz magnetic field due to house wiring, distribution lines, electric motors, etc., for individuals in the U.S. population is about 10^{-7} T [30], that is, orders of magnitude smaller than the earth's stationary magnetic field. Starting from this premise, the magnetosome in the cytoplasm was modeled as a damped harmonic oscillator with an external 60-Hz modulation [31]. The restoring force is the force pushing to align the magnetosome's moment with the earth's magnetic field, and the damping is due to the viscosity of the cytoplasm. The associated equation is easily solved. Using reasonable values for the involved parameters, it is was found that even with exposure to a 60-Hz field with an amplitude of 5 μT, the alternating field transfers an amount of energy to the magnetosome that is orders of magnitude smaller than kT. In other words, the thermal agitations in the rotation far overwhelm any "signal" from an ambient 60-Hz field. But subsequently, the legitimacy of a simple linear approximation was questioned [32]. It was pointed out that there are intricacies that make the viscosity of the cytoplasm, which determines the damping coefficient in the model, hard to specify. Most importantly, the possibility of many individual magnetosomes in a cell acting in concert should be considered. With N magnetosomes in a cell instead of just one, the signal-to-noise ratio is $\sqrt{N}$ times larger. The explanation for this apparent amplification is the same as with the aforementioned N ion channels in the shark's electroreception. An alternative model that includes such cooperativity leads to a signal-to-noise ratio that is well over unity with a 2-μT amplitude 60-Hz magnetic field [32]. However, almost nothing is currently known about how forces on magnetosomes are transduced into physiological signals. More solid estimates of detection thresholds can probably be derived only after such biophysical mechanisms are revealed.

Electric fields are also of interest. Close to a power line, a human can be exposed to an electric field of about 10 kV/m. Two steps have to be taken to get to an assessment of the transmembrane voltage that such an exposure leads to. First of all, living tissue is much more conducting than air. So, charge in the tissue will move and follow the external field until it is compensated. Depending on the amount of movable dipoles, different materials

have different dielectric permittivities. The ratio between the internal field and the field in the air is [33,34]:

$$\frac{E_i}{E_0} \approx \varepsilon_0 \omega \rho_t \tag{7.4}$$

Here $\varepsilon_0 = 8.8 \times 10^{-12}\ \mathrm{C^2/(N\,m^2)}$ represents the dielectric permittivity of a vacuum, ω is the angular frequency ($2\pi f$), and ρ_t is the resistivity of the tissue. So for a frequency of about 100 Hz and with a typical tissue resistivity of about 1–2 Ω m, the attenuation factor for the field entering the body is found to be in a range of 10^{-8}–10^{-7}. Hence, most of the external field goes around the person in the way water in a river flows around a big rock. Once inside the tissue, an amplification at the cell membranes occurs again through the mechanism explained in the previous paragraph. For a spherical cell with a diameter of about $d = 10\,\mu\mathrm{m}$ in a field E, the voltage across the diameter will be $\Delta V = Ed$, and the eventual field in the membrane will be of the order of $E_{\mathrm{mem}} \approx E(d/h)$, where h is the thickness of the membrane. With $h \approx 5\,\mathrm{nm}$ we find an amplification factor of about a 1000. We thus find a net conversion factor of 10^{-5}–10^{-4} and an electric field of about 0.1–1.0 V/m across a membrane as a result of the 10-kV/m power line exposure. This leads to an ELF-induced potential difference of at most 10^{-8} V across the membrane. It should, however, be noted that muscle cells or nerve cells are cylindrically shaped and may have lengths in the millimeter or even centimeter range. When the imposed field is along the axis of the cylinder, there may be a conversion factor at the caps of the cylinder that is two to three orders of magnitude higher.

When a living cell is suddenly exposed to an external electric field, ions will start flowing in the conducting interior to compensate for this field. In a typical mammalian cell, it is generally within microseconds that ions have accumulated near the membrane to achieve a zero intracellular electric field. This means that stationary electric fields and ELF (<300 Hz) AC fields distribute over cell membranes. Power lines and high-voltage distribution stations have been the subject of a lot of public anxiety. The power grid operates at 60 Hz in the United States and at 50 Hz in most other countries, that is, well within the ELF regime.

The 10^{-8} V that we derived may appear small relative to, for instance, the transmembrane potential of about 0.1 V that is present in about every living cell. However, when we talk about detectability, this 10^{-8} V should first be compared to the transmembrane voltages due to Brownian motion. The thermal noise voltage across standard resistors was already detected in the 1920s [35]. A formula was subsequently derived by Nyquist [36]:

$$\langle dV^2 \rangle = 4kTRdf \tag{7.5}$$

This equation gives the average square voltage in a frequency window of width df. The noise is white, that is, it has the same intensity at all frequencies. Technically, this would lead to an absurdity. It would imply that the noise carries an infinite amount of energy. However, as Nyquist already pointed out, $\langle dV^2 \rangle$ starts vanishing when we get to high frequencies f where $hf \approx kT$. Here, h represents Planck's constant, $h = 6.6 \times 10^{-34}\,\mathrm{J\,sec}$. At these high frequencies, quantum physics takes over and makes $\langle dV^2 \rangle$ go to zero. Such high frequencies are not in our realm of interest.

What Nyquist had in mind for a resistor in his derivation was a Brownian gas of frequently colliding charge carriers. With a 5-nm cell membrane that consists of a lipid bilayer with embedded proteins, the charge carriers are small ions (Na^+, K^+, Cl^-, etc.).

The ions do not form a "gas" inside the membrane, and it is not *a priori* obvious that Nyquist's formalism would apply. The equilibrium noise current through a membrane that separates two ionic solutions is due to two-sided shot noise. Shot noise was first described by Schottky [37] in the context of vacuum amplifier tubes. It is due to the elementary charge being finite and the charge carriers making random "jumps." It can be shown that two-sided shot noise ultimately leads back again to Nyquist's Equation 7.5 [38,39]. Ultimately, Equation 7.5 is a manifestation of something much more general than Nyquist may have had in mind. What underlies Equation 7.5 is Einstein's fluctuation–dissipation theorem. This theorem says that the same random collisions that cause diffusion, thermal noise, or shot noise also cause dissipation, friction, or resistance. The theorem, moreover, makes this connection quantitative:

$$\beta = \frac{kT}{D} \tag{7.6}$$

For the motion of a macromolecule in a liquid, D is the diffusion coefficient and β is the coefficient of friction, that is, the ratio $\beta = F/v$, where F represents the pulling force and v represents the resulting average speed. But in the context of the current through a membrane, β represents the electrical resistance ($R = V/I$). For D we find $D = e^2 P_S c$ in the membrane electrical case. Here, P_S is the membrane permeability to the monovalent ion S that is responsible for the current, c represents the concentration of this ion on both sides of the membrane, and e is the elementary charge.

Electrically, a cell membrane can be modeled as in Figure 7.1a. A lipid bilayer membrane has a capacitance of about 1 $\mu F/cm^2$. The capacitance of an actual cell membrane is generally not much different. The resistance of a pure lipid bilayer depends on the ionic concentrations of the solutions on either side of the membrane. With these concentrations at biological levels the resistance of a lipid bilayer membrane can be as high as $10^9\ \Omega\,cm^2$. Because of the presence of ion channels, ion transporters, and ion pumps [40,41], an actual cell membrane has a resistance that is orders of magnitude smaller (typically about $10^3\ \Omega\ cm^2$). The resistance of a patch of membrane is inversely proportional to the area of that patch. So, in order to characterize a membrane, the approach is to measure the resistance through an actual patch and then multiply it with the surface area of that patch. That is why we give the resistance of a membrane in terms of $\Omega\ cm^2$.

The setup in Figure 7.1a is equivalent to the one in Figure 7.1b, that is, an ordinary RC circuit. When calculating the characteristic time, RC, of the circuit, the surface area cancels

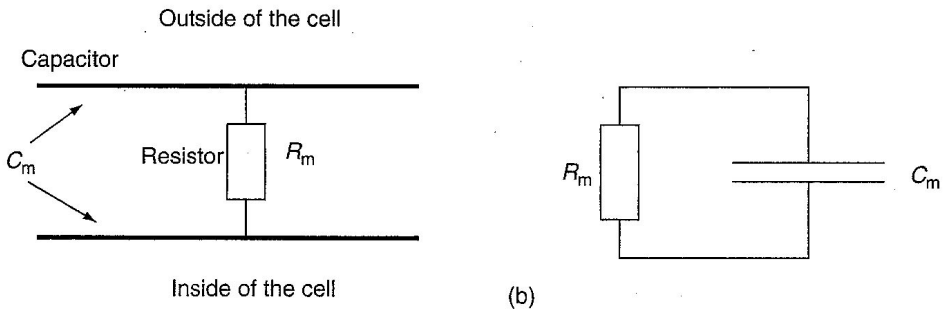

FIGURE 7.1
The electrical structure of the membrane is shown on the left. R_m and C_m are the resistance and capacitance between the inside and outside of the cell, respectively. The resistor also provides a thermal electromotive force. The equivalent circuit is shown on the right.

out. For a pure lipid bilayer the *RC* time constant can be of the order of minutes. But for a cell membrane it is of the order of milliseconds.

In our context, the resistor in Figure 7.1 is not just a resistor, but, following Nyquist (cf. Equation 7.5), also a white noise generator. At each frequency the resistor generates a harmonic oscillation. All these harmonic oscillations have the same amplitude. To evaluate the voltage across the capacitor, we thus have to analyze a simple *RC* circuit with an AC source. It has been argued that the high frequencies, that is, $f > (RC)^{-1}$, that are generated in the resistor do not have enough time to build up across the capacitor [42]. However, for low-frequencies, that is, $f < (RC)^{-1}$, changes are sufficiently slow for the capacitor to keep up and follow the voltage in the resistor. In this view the transmembrane voltage is the voltage across the capacitor, and the equilibrium noise is thus expected to occur mostly at low-frequencies. As mentioned before, the *RC* time of a cell membrane is of the order of milliseconds, and ELF fields thus operate in the $f < (RC)^{-1}$ regime where the noise is largest. A straightforward quantitative analysis shows that the low-frequency equilibrium noise far overwhelms any reasonable ambient power frequency field [42]. There would be no way to ever instantaneously detect such a field.

It was later put forward that everything that is happening in the cell membrane should, in the model of Figure 7.1, be imagined to happen inside the resistor [43]. Membrane proteins go through their catalytic cycle against a background of intramembrane noise. Inside the membrane means, in the context of Figure 7.1, inside the resistor. In this picture, the thermal noise voltage (cf. Equation 7.5) derives from a net electric field that results from inhomogeneities in the distribution of the charge carriers. Now at low-frequencies, the capacitor will be able to follow the imposed oscillation and effectively produce a field to counter the field generated inside the resistor (Figure 7.1a). This model thus leads to a vanishing net potential inside the membrane at low-frequency. At high frequency, the voltage changes in the resistor are too fast for the capacitor to keep up with. The capacitor will remain uncharged, and the thermal AC voltage will not be compensated for.

However, Figure 7.1 is no longer the appropriate model when we try to derive the intramembrane electric fields. For a cell of about 20 μm in diameter, the surface area amounts to about a billion square nanometers. The membrane is only about 5 nm thick, so the resistor resembles a very thin sheet. The lateral conductivity, that is, the conductivity from one place on the sheet to another, is very low. So at different spots on the sheet, different unrelated noise fields are generated. The more sensible model would therefore be one where the resistor in Figure 7.1 is cut up into millions of independent parallel resistors. Each of these resistors creates its own field. The capacitor plate corresponds to the conducting liquid on either side of the membrane, and it can be conceived of as having perfect lateral conductivity. So each resistor generates its own particular field, but they all experience the same field from the capacitor. With this model the noise gets very large. Not only there are more, say *N*, resistors producing noise. Each of these resistors has a resistance *NR* (*N* parallel resistors of resistance *NR* lead to a net resistance of *R*) and, according to Equation 7.5, thus produces more noise. Because the *N* parallel resistors that make up the resistance *R* are independent, they oscillate out of phase at each frequency *f*. As a result the parallel resistors end up pushing a lot of current in and out of each other. Most of the generated noise current thus remains intramembrane and never reaches the capacitor. The mathematics associated with this parallel setup is challenging, but an exact solution can be derived [39,44]. The capacitor, and therefore the *RC* time, plays no role in the intramembrane noise. The intramembrane noise is white and has an intensity that is many orders of magnitude larger than the noise that reaches the capacitor. What matters for biological function is actually the intramembrane noise. This, after all, is the noise that a membrane-embedded protein would "feel." The protein's catalytic cycle takes place

against the background of such noise. The parallel setup model leads to a noise intensity that is much larger than that of the earlier models.

At first sight, all this extensive treatment of intramembrane noise may seem to have little to do with the two-sided shot noise that a membrane is subject to. However, when rigorously modeling the membrane as a thin sheet in an ionic solution, something similar to the overwhelming intramembrane noise is found. The ions that constitute the net charge on the membrane in Figure 7.1a move across the membrane–solution interface with an average speed of about 100 m/sec. This is just their thermal motion, and it is easily derived from $(1/2)mv^2 \approx kT$. This effectively causes laterally traveling electric pulses in the membrane. The noise intensity of these traveling electric pulses appears to be many orders of magnitude higher than the noise that is due to the shot noise-like membrane passages by the ions [39].

Current models of membrane noise thus lead to transmembrane voltage noise estimates that far exceed the strength of any reasonable magnitude ELF field-induced "signal." What the previous paragraphs lead up to is the conclusion that an ELF signal cannot be detected instantaneously.

However, under certain conditions and given enough time, even the smallest signal can get out of the noise band. The following example is meant to illustrate this. Consider the system depicted in Figure 7.2. Let the the resistance R represent a membrane patch. For simplicity, imagine that on either side of the resistor there is an infinite reservoir (i.e., a capacitor with infinite capacitance), so no net voltage can develop across the resistor. The average square charge $\langle q^2(t)\rangle$ that accumulates on either side of the membrane can be easily derived from Equation 7.5 and amounts to

$$\langle q^2(t)\rangle = \frac{2kT}{R}t \tag{7.7}$$

Again, there is an obvious analogy between Equation 7.7 and the well-known diffusion formula $\langle x^2(t)\rangle = 2Dt$, which describes the average square displacement of a particle with a diffusion coefficient D during a time interval of length t. The above formula clearly shows how, in an electrical context, kT/R plays the role of the diffusion coefficient D.

From Equation 7.7 we infer that for the accumulated charge as a function of time we have $|q_{Br}(t)| \approx \sqrt{\langle q^2(t)\rangle} \propto \sqrt{t}$. The thermal noise-driven accumulation of any charged or uncharged molecule on either side of the membrane carries this $\sqrt{t}$ proportionality. The coupling of ELF electromagnetic fields to biochemical activity occurs mostly through

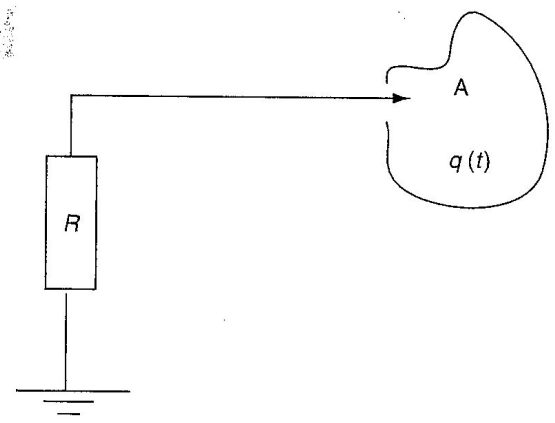

FIGURE 7.2
A resistor is connected to the ground and to an infinite reservoir A. The net voltage between the reservoirs remains zero. The situation is like the one in Figure 7.1 with the capacitor having infinite capacitance. Because of Brownian motion of electrons in the conduction band, there is a zero-average fluctuating current through the resistor. The net charge accumulating in the reservoir A is the result of these fluctuations in the same way that diffusive displacement is the result of random Brownian kicks. We have $\langle q^2(t)\rangle = 2(kT/R)t$ for the average square charge accumulation in time t.

membrane proteins. Membrane proteins whose conformational changes involve significant changes of the dipole moment are particularly sensitive. ELF fields can affect the catalytic rates of such proteins. So, for instance, electrogenic ion pumps [41], and also transporters or pumps that just carry a dipole, may have a slightly altered throughput in the presence of an ELF field. If there is no restoring force for a transported or pumped molecule, the accumulation will continue. The cumulative effect of the altered throughput will be a linear function of time. The excess charge that accumulates because of an ELF field thus follows $q_{\mathrm{ELF}} \propto t$.

Consequently, we see that on a small timescale the Brownian noise ($\propto \sqrt{t}$) will be stronger than the signal ($\propto t$). But there will always come a time $t = t_*$ when $|q_{\mathrm{Br}}(t_*)| = |q_{\mathrm{ELF}}(t_*)|$, and we then achieve S/N = 1. It depends on the values of the proportionality constants when t_* occurs. If molecular change is the measurement criterion, then it is only on timescales of the order of t_* that the effect becomes measurable. Estimates for t_* with realistic ELF exposure have been made [45] and have led to a timescale larger than the age of the universe.

7.6 Nonequilibrium Noise

In the previous section, we considered equilibrium noise. A living cell, however, constitutes a system that is far from equilibrium. Between the intracellular and extracellular solutions there is an electric potential difference of about 100 mV. For ions like Na^+, K^+, Cl^-, and Ca^{2+} there is a more than tenfold difference between intra- and extracellular concentration. The 100-mV transmembrane voltage over a width of about 5 nm implies a very strong field of tens of megavolts per meter.

The electrochemical potential across the cell membrane is an energy source for many processes [41]. The Na, Ca exchanger, for instance, is a membrane protein that picks up a sodium ion on the outside and then goes through a cycle in the course of which it drops the sodium ion off on the inside. The protein couples the energetically downhill movement of sodium to the uphill transport of calcium. In the course of the cycle a calcium ion is picked up on the inside and pumped, against the electrochemical potential, to the outside. The membrane potential is maintained by ATP-driven ion pumps. The most common of these is Na, K-ATPase. This is a membrane protein that, in the course of its catalytic cycle, hydrolyzes one ATP and uses the released energy to transport three sodium ions out of the cell and bring two potassium ions in.

Each working protein is like a small engine. A living cell contains millions of these engines: they are continuously converting energy from one form to another, and in the process, they are also generating heat, that is, dissipating energy. A living cell constitutes a far from equilibrium system, and the continuous transduction and dissipation of energy generates noise, which adds to the thermal, Brownian noise that was discussed in the previous section.

It would not be against the first law of thermodynamics (i.e., conservation of energy) if ion pumps were to extract heat from the environment and use it to power the maintenance of the transmembrane potential. This would, however, be in gross violation of the second law of thermodynamics. There are many equivalent formulations of the second law. The most common formulation is the proposition that every isolated system strives to increase and maximize its entropy. The teleological form of this formulation is somewhat bewildering. After all, most laws in science are formulated as conservation laws, for

example, conservation of energy, or as causal laws, for instance, Newton's $F = ma$. However, after properly defining entropy, entropy maximization is often the easiest form of the second law to work with when dealing with macroscopic systems.

When going to the molecular realm, the second law can pose some challenging paradoxes. Consider, for instance, an ion channel in a cell membrane. Many ion channels rectify, that is, they pass current more easily in one direction than in the other. So the I–V characteristic is not a straight line through the origin, but it also has a curvature. Any frequency from the white spectrum of equilibrium noise should, in principle, be rectified. It thus might look like a rectifying ion channel could use zero-average equilibrium Nyquist noise to charge a battery. It would not work, of course. As pointed out above, it would be in violation of the second law. Thinking in the context of rectifying p–n junctions, solid-state physicists ran into this paradox long before ion channels were discovered. In 1950, L. Brillouin wrote a paper "Can the Rectifier Become a Thermodynamic Demon?" [46]. In this paper, he presents a short derivation to show that in a circuit with all components at the same temperature, no diode can rectify. He is aware that his case represents a special case of the so-called principle of detailed balance: "No system in thermal equilibrium in an environment at constant temperature spontaneously and of itself arrives in such a condition that any of the processes taking place in the system by which energy may be extracted, run in a preferred direction, without a compensating reverse process." The principle is a consequence of the second law [47,48] and, for our rectifier, basically states that there must, on average, be as much current in one direction as there is in the opposite direction.

In the *Feynman Lectures on Physics* [49] a ratchet and pawl system, originally thought up by Smoluchowski [50], is considered and eloquently discussed. The device operates as a mechanical rectifier (Figure 7.3) and essentially establishes the mechanical equivalent of Brillouin's paradox. The paradox is solved with the realization that the pawl must also be

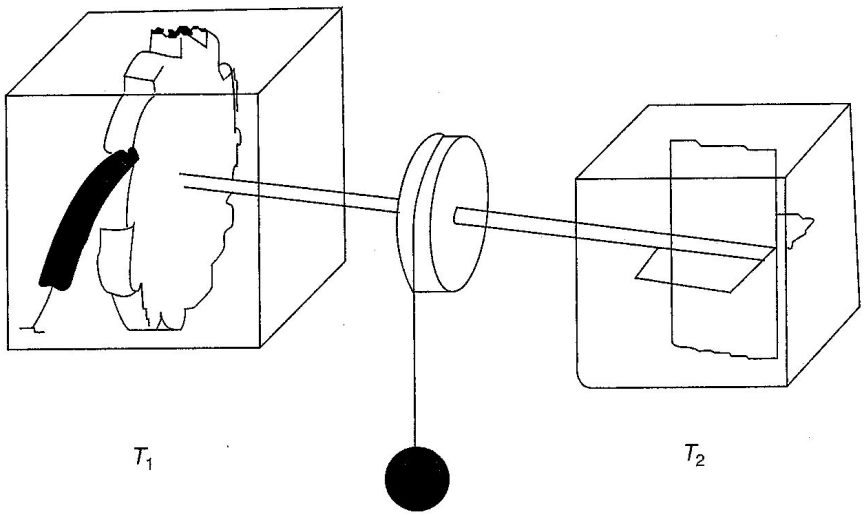

FIGURE 7.3
The mechanical thermal ratchet as it was originally conceived by Smoluchowski [50] and later discussed by Feynman et al. [49]. The device is small, and the paddle wheel in the right reservoir is moved by collisions of the molecules from the surrounding medium against the paddles. Because of the asymmetry of the teeth, the ratchet and pawl in the left reservoir allow motion in one direction and block it in the opposite direction. With the resulting net rotation it should be possible, in principle, to lift a weight. However, it would be in violation of the second law of thermodynamics to extract work from thermal fluctuations in the equilibrium situation, that is, $T_1 = T_2$. The solution of the paradox lies in the realization that the ratchet and pawl are also subject to thermal fluctuations if the system is small.

subject to thermal noise. The pawl involves a spring, and the spring will, at thermal equilibrium, exhibit a Boltzmann distribution over the accessible energy range. Even here the second law is involved, though on a deeper level. Given the macroscopic variables (e.g., temperature, concentration, pressure, etc.) there are still many possible molecular arrangements, that is, microstates, that correspond to that macrostate. For a fixed amount of energy the Boltzmann distribution is the energy distribution that has the most permutations [20]. It is therefore the most likely distribution. On the level of statistical mechanics, the second law can be formulated as the rule that given a macrostate, every microstate that corresponds to that macrostate has equal probability.

Second law issues can be subtle. The connection between statistics, entropy, information, and physical work still poses paradoxes that are hard to fathom. Books and articles still appear in which researchers are attempting to come to a fuller understanding and a better intuition [51,52]. At the scale of ion channels the simple invocation of detailed balance reveals little. An appropriate description is like the one Feynman gave for his mechanical ratchet and pawl: it involves Boltzmann distributions and Brownian motion. So it would simply be wrong to take any frequency from the white spectrum of equilibrium noise and model a rectifying ion channel as subject to this oscillation. The ion channel itself and its Brownian fluctuations have to be included in the description. At equilibrium, no part of a system can be "subject" to any other part. This is what detailed balance can be interpreted to mean.

However, when energy is dissipated, it is possible for one part of the system to impose its fluctuations on another part. When a rectifying ion channel is subject to nonequilibrium fluctuations, it will actually rectify the fluctuations and drive a net current. Consider, for instance, an electrogenic ion pump like Na,K-ATPase. As was mentioned before, this pump utilizes the energy of ATP hydrolysis to pump three sodium ions out and pump two potassium ions in. All this transport is against the electrochemical potential and requires about $15kT$ units of energy per stroke under physiological conditions. The power source is the hydrolysis of ATP, which under physiological conditions, releases about $20kT$ units of energy per cycle. It is the remaining $5kT$ that drives the process forward and that is ultimately released as heat. Na,K-ATPase is binding and releasing ions and thus generates fluctuating electric fields in its direct vicinity. For a nearby ion channel these fields can be conceived of as imposed because the $5kT$ that drives the Na,K-ATPase cycle is enough to overwhelm the small amount of energy ($<1kT$ [53]) necessary for the opening or closing of a channel. There is no feedback from the channel to the pump. The channel will rectify the fluctuations as a result, and a zero-average field can thus lead to net charge transport. In essence, the nonequilibrium fluctuations generated by the pump and imposed on the channel are part of the conversion of chemical energy, that is, the energy in ATP, to an electrochemical potential across the membrane.

So energy-dissipating, nonequilibrium oscillations and fluctuations are able to do work. ELF radiation from outside the organism can impose a varying field on an ion channel in much the same way that the nearby ion pump from the previous paragraph can impose a field on an ion channel. ELF radiation brings energy into the organism. Part of this energy will be dissipated to become heat, and part of it may be converted into chemical or electrical work. There is obviously no feedback from an ion channel back to the ELF source.

The selectivity of ion channels for the different kinds of ions is still hard to understand and model. But the rectification property is much easier to intuit (Figure 7.4). The channel is shaped like an asymmetric double cone, and charges in the lining of the channel are

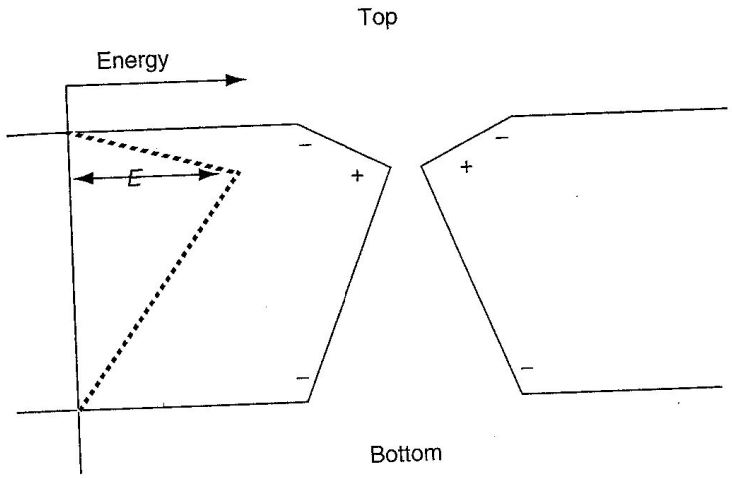

FIGURE 7.4
A simple continuum model of an ion channel imagined to be shaped like an asymmetric double cone. The energy profile on the left depicts the activation barrier that a positive ion going through the channel has to pass. The barrier has an obvious anisotropy.

indicated in the figure. A sodium or potassium ion that is going from the top to the bottom of the channel faces a rapid increase of the potential and then a slow decrease. A sodium or potassium ion that goes through in the opposite direction faces a slow increase and a fast subsequent decrease. A positive ion thus has a larger force to overcome when going from top to bottom than when going from bottom to top. Because of this, an imposed zero-average oscillation will lead to a net current [54]. As a matter of fact, any anisotropic potential shape along the length of the channel will rectify a zero-average harmonic field to lead to a net current [55]. Ion channels are proteins consisting of many amino acids, and anisotropy along the inside lining will be the rule rather than the exception.

The plethora of ratchet research in the late 1990s has made it clear that almost any zero-average oscillation or fluctuation imposed on a ratchet-like structure as in Figure 7.4 leads to a net current. Imagine, for instance, a temperature oscillation. With energy expressed in units of kT, a variation in temperature implies an oscillation of the barrier height E. Because of the difference in relaxation times on the slopes on either side of the barrier, a net current will result [55–58]. Recently, ever more examples have been found of nature exploiting ratchet effects for the purpose of regulation [59].

Researchers have meanwhile also succeeded in making artificial channels. Cone-shaped (and therefore anisotropic) channels form when a heavy ion is shot through an artificial membrane [60,61]. The I–V characteristic for the current of different types of ions has subsequently been recorded. It has even been experimentally shown that net charge transfer results when a zero-average field is imposed on such an artificial channel. The channel is thus made to behave like a kind of pump that converts an AC input into a DC output [62].

Imagine a number of identical anisotropic channels in a vesicle with an otherwise impermeable membrane. Next, put a large number of such vesicles in a beaker with an ionic solution. Any nonequilibrium fluctuation from the environment, or any "signal" for that matter, will now be picked up and converted into an electrochemical potential. The convection caused by a temperature gradient will heat up and cool down the vesicles and lead to their electrically charging up. The electric component of an ELF electromagnetic field will do the same thing. The beaker could thus be a battery that recharges by harnessing any incoming nonequilibrium fluctuation. This mechanism might, moreover, have played a role in the emergence of early prokaryotic life.

The Fourier spectrum of the noise that is associated with processes that dissipate energy is not white. Nonequilibrium noise appears to have higher amplitudes at lower frequencies; in other words, it exhibits an intensity that decreases with frequency. The so-called $1/f$ noise was first studied in the 1920s in the very nonequilibrium context of thermionic vacuum tube amplifiers [37]. In current scientific discourse the term "$1/f$ noise" actually applies to all noises that have spectral densities behaving like $1/f^{\alpha}$, where α ranges from about 0.5 to about 1.5. Especially in electrical devices, such noise is very commonly and easily observed. It is also known as "excess noise" or "flicker noise." In a log–log plot the $1/f^{\alpha}$ behavior usually extends over several frequency decades.

In the 1930s, it was proposed that the flicker noise originated from a variable number of electrons present in the conduction band. Electrons would shuttle between a free state and a bound state as in a chemical reaction. Let the relaxation time of that reaction be $1/\lambda$. This leads to a simple exponential relaxation $N(t) = N_0 \exp[-\lambda t]$ after any kind of fluctuation that has a magnitude N_0. The Fourier transform of the exponential decay is easily found:

$$F(\omega) = N_0 \int_{t=0}^{\infty} \exp\left[-(\lambda + \mathrm{i}\omega)t\right] \mathrm{d}t = \frac{N_0}{\lambda + \mathrm{i}\omega} \tag{7.8}$$

For the power spectral density, $S(\omega) = \|F(\omega)\|^2$, we find:

$$S(\omega) \propto \frac{1}{\lambda^2 + \omega^2} \tag{7.9}$$

where the proportionality constant involves the magnitudes of the fluctuations as well as the rates at which fluctuations occur. The power spectral density is a useful quantity as it describes how the energy in the noise is distributed over the different frequencies. $S(\omega)\mathrm{d}\omega$ is proportional to the amount of power that the noise carries between the frequencies ω and $\omega + \mathrm{d}\omega$. Equation 7.8 describes a so-called Lorentzian power spectrum. With a log scale for the frequency, the resulting curve is a sigmoid. At high ω, $S(\omega)$ behaves like $1/\omega^2$. As better data became available, it was found that a better fit was obtained when a distribution of infinitely many relaxation times was assumed [63]. Take, for instance, a uniform distribution of relaxation times between λ_1 and λ_2. With Equation 7.9 this leads to:

$$S(\omega) \propto \frac{1}{\lambda_2 - \lambda_1} \int_{\lambda_1}^{\lambda_2} \frac{1}{\lambda^2 + \omega^2} \mathrm{d}\lambda = \frac{1}{\omega(\lambda_2 - \lambda_1)} \left\{ \arctan\frac{\lambda_2}{\omega} - \arctan\frac{\lambda_1}{\omega} \right\} \tag{7.10}$$

It is easy to check that on $\lambda_1 < \omega < \lambda_2$ this $S(\omega)$ is approximately proportional to $1/(\omega(\lambda_2 - \lambda_1))$. This $S(\omega)$ is, moreover, roughly constant for $\omega < \lambda_1$ and drops off like $1/\omega^2$ when $\omega > \lambda_2$.

If we let, between λ_1 and λ_2, the relaxation rates contribute proportional to $\lambda^{-\beta}$, we can actually get any $1/f^{\alpha}$ dependence that we want, since

$$S(\omega) \propto \int_{\lambda_1}^{\lambda_2} \frac{1}{\lambda^{\beta}(\lambda^2 + \omega^2)} \mathrm{d}\lambda \propto \frac{1}{\omega^{1+\beta}} \quad \text{for } \lambda_1 < \omega < \lambda_2 \tag{7.11}$$

At $\omega < \lambda_1$, this spectrum would again flatten out.