W-PM-WS1-1

THE CRAYFISH MECHANORECEPTOR: A BIOLOGICAL EXAMPLE OF STOCHASTIC RESONANCE

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In linear information theory, electrical engineering and neurobiology, random noise traditionally has been viewed as a detriment to information transmission. *Stochastic Resonance* (SR) refers to a nonlinear, statistical dynamics whereby information flow in a multistate system is *enhanced* by the presence of optimized, random noise. A major consequence of SR for signal reception is that substantial improvements are possible in the detection of weak periodic signals. Although SR has recently been demonstrated in several carefully prepared physical systems, it may also occur naturally, and an intriguing possibility is that biological systems may have evolved the capability to exploit SR by optimizing endogenous sources of noise. Sensory systems are an obvious place to look for SR, since they excel at detecting weak signals in a noisy environment. We have demonstrated SR for the first time in a biological system: the crayfish mechanoreceptor cells. This talk discusses SR by comparing the biological data with that obtained from electronic FitzHugh-Nagumo and simple threshold models. Our results show that individual neurons can provide a physiological substrate for SR in sensory systems.

W-PM-WS1-2

WHY SENSORY NEURONS "NEED" TO BE NOISY?

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In the peripheral nervous system, there are several modalities of encoding used by receptors to write sensory inputs as intervals between action potentials. The range of propagation speeds at which nerve fibers transmit that information to the central nervous system is also large. Why? Focussing on well known nonlinear properties of action potential propagation in nerve fibers, in this talk I discuss theoretical results showing that, unless noise is considered a active player, sensory information (Shannon's) written at a given receptor level (as consecutive inter-spikes intervals) decay always as a function of travelled distance. The rate of information loss is a power law of the distance travelled where the exponent depend both on the recovery of excitability of the nerve fiber, and on the maximum speed of action potentials propagation. Paradoxically, information losses are minimized for very small sensory inputs entering the receptor in the presence of noise; while mayor distortion occurs for large sensory inputs in the absence of noise. Simple analysis of the theory tell us how noisy neurons take advantage of variability (spatial and temporal) and also where and why very fast myelinic nerve fibers or very slow amyelinic ones are "selected" by mother nature to connect the brain with the sensory world.

W-PM-WS1-3

FLUCTUATIONS AND NOISE IN THE VISUAL SYSTEM

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Neurons are rarely silent. In the absence of stimulation they usually produce continuous, seemingly stochastic, activity. In addition, the responses to repeated presentations of a stimulus are variable, and fluctuate around a mean response level. This variability is surprisingly large. In order to understand information processing in the nervous system it is important to determine the nature, source and possible function of such fluctuations ("noise").

The random barrage of synaptic events is widely thought to account for much of the noise in the nervous system. In the visual system, an additional important source of noise is believed to be the quantal fluctuations of light.

Recent experiments on retinal ganglion cells and on thalamic neurons of cats and monkeys suggest that (at least part of) the variability in the spike discharge of these neurons originates in the neurons themselves, and not in their synaptic or photic input. Below the level of the cortex this noise is additive.

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W-PM-WS1-4

NOISE IN SENSORY AND SYNAPTIC CODING. A SURVEY OF ITS HISTORY AND A SUMMARY OF ITS CONCLUSIONS.

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Numerous biological publications have noted that natural perturbations are ubiquitous and relevant to neural function, analyzing theoretically and experimentally the essential consequences of noise (see Segundo J.P. et al., The inevitability of noise and its influence on sensory and synaptic coding. Proc. Second Appalachian Conference on Behavioral Neurodynamics, in the press). In sensory physiology Spekreijse and collaborators demonstrated in the '60s that noise benefitted transduction, a seminal discovery soon confirmed and extended by publications in the '70s and '80s. In synaptology, the issue of regularity vs. noise has been discussed for years. Demonstrated convincingly were the essential facts of the striking differences between clean and noisy drivings, that differences assume several forms, and that consequences are contingent on the stimulus-noise relation. A contemporary renewal of interest includes relevant biological observations dealing with receptors or synapses.

W-PM-WS2-1

PHOTOSYNTHETIC REACTION CENTERS DYNAMICS VIA ULTRAFAST INFRARED SPECTROSCOPY

((B. R. Cowen, G. C. Walker, S. Maiti, C. C. Moser, P. L. Dutton, and R. M. Hochstrasser)) Department of Chemistry and Johnson Research Foundation, University of Pennsylvania, Philadelphia, PA 19104

Ultrafast transient infrared spectroscopy is a new technique that allows one to monitor both active site and protein changes in biomolecules. Previous ultrafast spectroscopies have been limited to monitoring active site changes. The ultrafast transient infrared (IR) absorption following optical excitation of *Rb. sphaeroides* R-26 reaction centers in the amide and carbonyl spectral region, 1550-1750 cm^{-1} was measured. To accomplish this a transient IR spectrometer based on Ti:Sapphire with 300 fs time resolution, tunable from 1550-2000 cm^{-1} and having 10^{-5} OD sensitivity was developed. The IR transient absorption was measured in regions where BChl/BChl$^-$ shows static absorption differences in model systems, in search of clear spectral evidence for the chlorophyll monomer in the electron transfer process. Our results did not show evidence for the electron residing on the chlorophyll monomer. Recent results with interpretations will be presented. The ability to monitor the protein directly with chemical bond resolution on an ultrafast timescale makes ultrafast infrared spectroscopy a powerful tool to study protein dynamics.Sponsored by NIH grants to R.M.H. and P.L.D.

W-PM-WS2-2

TRANSIENT IR SPECTROSCOPY IN THE NSEC-TO-SEC TIME RANGE WITH TUNABLE DIODE LASERS: PROTOLYTIC REACTIONS IN BACTERIAL PHOTOSYNTHETIC REACTION CENTERS

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Proton transfer in photosynthetic reaction centers (RC) is coupled to light-induced electron transfer on the level of the secondary electron acceptor quinone Q_B. Upon transfer of the first electron to form Q_B^-, a proton is taken up by the RC, although probably not bound by the quinone as Q_BH. Transfer of the second electron then leads to formation of Q_BH_2, in a sequence of e^- and H^+ transfer which is presently undetermined. We have studied the protolytic reactions in *Rb. sphaeroides* RC by transient IR spectroscopy in the nsec-to-sec time domain. Continuous IR radiation from tunable lead salt diode lasers was used to follow IR transmission changes in the 1780 to 1420 cm^{-1} range caused by protolytic reactions at ASP or GLU side chain residues (1750-1700 cm^{-1}), protein backbone rearrangements (amide I: 1680-1620 cm^{-1}; amide II: around 1560 cm^{-1}), and further modes from amino acid side chains and from the quinones. Very small IR transients ($\Delta A/A \approx 10^{-4}$ to 10^{-3}) in the 1700-1760 cm^{-1} range were found to arise from protonation changes at ASP/GLU residues near Q_B, the major signal at 1725 cm^{-1} being due to H^+ uptake by GLU L212. This set of deprotonation/protonation reactions reflects a *dynamic electrostatic reaction field* around Q_B which balances the excess negative charge upon Q_B^- formation and may serve to provide the protons for the formation of Q_BH_2 upon transfer of the second electron.

W-PM-WS2-3

TIME-RESOLVED STEP-SCAN FOURIER TRANSFORM INFRARED SPECTROSCOPY APPLIED TO BIOLOGICAL SYSTEMS

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Static infrared difference spectroscopy is able to reveal structural changes in biological systems between states which can be stabilized. However, to study the dynamics of structural changes, time-resolved techniques are required. The method of step-scan time-resolved FT-IR spectroscopy is described at greater detail and the advantages and disadvantages are discussed. It is shown that, in contrast to other time-resolved FT-IR methods, the time-resolution only depends on the bandwidth of the IR detector and of the acquisition electronics. The requirements on the systems to be studied is especially emphasized. Results on the photoreaction of bacteriorhodopsin are shown for the ns and μs time-range. From the spectra, it can be concluded that there exists a KL/L equilibrium. Sterically altered chromophores are incorporated and their influence on the dynamics tested. Finally, the rebinding of photolyzed CO in CO-myoglobin is investigated with this method and results on structural changes of the protein caused by expelling CO from the binding site are demonstrated. The μs kinetics reveal the rotational diffusion of the CO molecule.

W-PM-WS2-4

WORKSHOP ABSTRACT
PROTEIN DYNAMICS AND INFRARED SPECTROSCOPY

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Even the simplest protein reaction is complex and is governed by protein motions. To follow the motions, markers in various regions of the protein must be followed over wide ranges in time, temperature, pressure, and external parameters such as viscosity and pH. Observations in the infrared are essential. Using myoglobin as example, I will show that the combined use of infrared difference spectroscopy, temperature-derivative spectroscopy (TDS), and flash photolysis leads to a deeper insight into the role of protein dynamics in the ligand binding process.

WORKSHOP 3: ADVANCES IN BIOLOGICAL FLUORESCENCE

W-PM-WS3-1

APPLICATIONS OF TOTAL INTERNAL REFLECTION FLUORESCENCE MICROSCOPY.

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Total internal reflection reflection fluorescence microscopy (TIRFM) is particularly useful for studying protein binding/unbinding kinetics and aggregation processes at cell surfaces or model membranes. We present a few examples of TIRFM relevant to membrane biophysics. The first is the measurement of the reversible binding of fluorescence-labeled cytoplasmic proteins (particularly protein 4.1) to erythrocyte and planar phosholipid membranes. This study combines TIRFM with photobleaching recovery techniques. The results suggest that a substantial (and possibly biologically significant) portion of the binding occurs directly onto phospholipids. This observation bears on the theoretical possibility that such "nonspecific" binding may guide or enhance specific binding to membrane anchors and thereby be relevant to the submembrane dynamics of the cell's mechanical and motile properties. Another example observes the formation and fine structure of acetylcholine receptor clusters on developing muscle cells in culture. It involves the combination of TIRFM with an image analysis technique based on spatial autocorrelation, which provides a quantitative measure of microcluster aggregation and periodicity. Supported by NIH NS14565 and NSF DMB8805296.

W-PM-WS3-2

FLUORESCENT PROBES OF FREE FATTY ACIDS.

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Fatty acids (FA) exist, *in vivo*, primarily bound to albumin. Although only a very small fraction of the total FA is present as the free molecule (FFA) in aqueous solution, it is this component of the total FA that is biologically active. Recently we have developed a fluorescent probe (ADIFAB) that is capable of quantitating the very low concentrations of the physiologically important long chain FFA. ADIFAB's design is analogous to the fluorescent probes of free metal ion concentrations. A fluorescently (acrylodan) modified intestinal fatty acid binding protein (I-FABP) that specifically binds the long chain FA, undergoes a change in emission wavelength and quantum yield upon binding a single FA. The ratio of fluorescence emissions at 505 and 432 nm, together with the measured equilibrium binding constants for each FA, is then used to determine the concentration of FA. Measurements of the binding properties of native FABPs from a variety of tissues and species have now been determined using the ADIFAB probe. The results of these measurements reveal large differences in FA binding constants for different FABPs. Moreover, the pattern of binding constants as a function of FA molecular species differ considerably among the different FABPs. Studies of the fluorescence spectroscopic properties of native and mutant proteins together with computer modeling provide a clear understanding of how protein structural changes are reflected in the fluorescence properties of ADIFAB. Moreover, these studies predict how FFA probes with significantly different FA binding and fluorescence response properties can be constructed. The properties of several of these newly constructed molecules will be discussed. This work is supported by NIH grant GM46931.

W-PM-WS3-3

Tryptophan as a Structural Probe in Peptides and Proteins, Mary D. Barkley, Department of Chemistry, Louisiana State University, Baton Rouge, LA 70803

The amino acid tryptophan is a superb intrinsic probe of peptide and protein structure because its fluorescence is so sensitive to environment. A complex fluorescence decay is usually observed for single tryptophans in polypeptide chains, presumably reporting the heterogeneous microenvironment of the the indole chromophore. The environmental sensitivity derives from two sources: two overlapping electronic transitions with different polarity in the first absorption band and multiple nonradiative decay channels.

We have used constrained tryptophan derivatives to dissect the different nonradiative processes of indole. First, the complex fluorescence decays reflect ground-state heterogenity which leads to excited-state heterogeneity. Second, the nonradiative rate of indole includes contributions from several environmentally sensitive pathways: solvent quenching, excited-state proton transfer, and excited-state electron transfer. Solvent quenching refers to the major temperature-dependent nonradiative process that occurs in all indoles in protic solvent. Two types of excited-state proton transfer reactions occur in indoles at neutral pH in the presence of a strong proton donor: intramolecular and intermolecular. Intra- and intermolecular proton transfer rates can be estimated from photochmical reaction yields for H-D exchange at aromatic carbons. Excited-state electron transfer has not been detected directly, but inferred from solute quenching experiments and substituent effects. Preliminary studies suggest that the electron transfer rate is independent of temperature.

W-PM-WS3-4

BIOSYNTHETIC INCORPORATION OF TRYPTOPHAN ANALOGS IN PROTEINS: EXCITING PROSPECTS FOR PROTEIN INTERACTION STUDIES. ((A. G. Szabo[1,2], C. W. V. Hogue[2], and J. Brennan[2])) [1]Institute for Biological Sciences, National Research Council, Bldg. M54, Montreal Rd., Ottawa Canada, K1A 0R6, [2]Department of Biochemistry, University of Ottawa, 451 Smyth Rd., Ottawa Canada, K1H 8M5.

Protein-protein interactions are ubiquitous in biochemistry and biology and often play a key role in cellular function. The understanding of the molecular details of these interactions are important to the elucidation of their role in biological processes. NMR spectroscopy and X-ray crystallography can provide atomic details of the structure and interactions in proteins. Because of practical limitations many problems involving protein-protein interactions are not amenable to study by these methodologies. In many of the studies of the structure and dynamics of proteins and their interactions, the information required for a sufficient understanding of their relationship to the function of the system can be localized to selected segments of the protein. The intrinsic fluorescence of tryptophan (TRP) in proteins has been shown to be a convenient method to obtain selective and useful local structural information. However, TRP fluorescence may appear to be limited in studies of protein-protein interactions where there is more than one TRP residue in the interacting proteins. Recently we demonstrated that 5-hydroxytryptophan with its distinct spectroscopic properties could be used to investigate protein-protein interactions. We have also shown how using 7AW, 5HW, and 4FW in studies of tryptophanyl-tRNA synthetase gave new mechanistic details of its function. Ross and coworkers, appreciated the significant potential of this approach and incorporated 5HW into bacteriophage λcI repressor and studied its interaction with operator DNA. In this presentation highlights of photophysical studies of the TRP analogs and their use in correlating the interrelationship of the structure, dynamics and function of proteins will be discussed. (Financial Support from the Natural Science and Engineering Research Council of Canada is acknowledged.)

WORKSHOP 4: THEORY AND MOLECULAR DYNAMICS COMPUTATIONS OF THE LIPID-PROTEIN SYSTEM: INTERACTION WITH EXPERIMENTAL FACT

W-PM-WS4

W-PM-WS4

No abstracts submitted.

TRANSFORMATION/PROLIFERATION

W-Pos1

DO TIME VARYING WEAK MAGNETIC FIELDS CAUSE CELL TRANSFORMATION?

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Proliferation of chicken embryo fibroblasts (CEF) was assayed by colorimetric method ([3-(4,5-dimethylthiazol 2-yl)]-2,5 diphenyltetrazolium bromide, MTT test) after exposure to sinusoidally varying magnetic field (SVMF), for 24 hours. There is an increase in cell proliferation (up to 42% at 100Hz/7 gauss) after exposure to SVMF; furthermore we see a direct correlation between the intensity of the fields (7, 6, 5 gauss/100Hz) and the rate of cell proliferation. Changing the frequency (100, 60, 50Hz/ 7 gauss) showed a similar correlation, which in all cases was higher then that of the sham exposed cells.

Normal rat fibroblast cells (F111) were infected by minute virus of mice (MVM), known to have a cytopatic effect on transformed cells (E. Guetta, M. Mincberg, S. Mousset, C. Bertinchamps, J. Rommelaere and J. Tal (1990) J. Virol., 64, 458-462). Exposure of viraly infected cells to SVMF (100Hz / 7 gauss / 4 days) resulted in complete extinction of all exposed cells, while sham exposed cells exhibited a normal growth patern. Isolating viral DNA (Hirt assay) showed increased viral DNA (Southern Blot and Autoradiography) in exposed cells as compared to unexposed ones.

Primary or 'normal' cells exposed to SVMF seem to undergo cell transformation. The methods in the present study complement and strengthen those observed before (A. H. Parola, N. Porat and L. A. Kiesow (1993) Bioelectromagnetics, 14, 215-228).

W-Pos2

MOLECULAR SPECIES OF PHOSPHOLIPIDS IN A MURINE STEM CELL LINE RESPONSIVE TO ERYTHROPOIETIN. ((S. Clejan[1,3], C. Mallia[2], D. Vinson[1], and B. Beckman[2,3])) Tulane University School of Medicine, Depts. of Pathology and Laboratory Medicine[1] and Pharmacology[2] and Program in Molecular and Cellular Biology[3], New Orleans, LA. 70112

Hematopoietic growth factors such as erythropoietin (Epo) are known to stimulate the proliferation and differentiation of target stem cells through the generation of lipid signalling molecules such as diacylglycerol (DG) and phosphatidic acid (PA). The kinetics of these responses are both rapid and transient while others are slower and sustained. In this study the molecular species of DG, phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI) and PA were analyzed in a murine stem cell line (B6SUt.Ep) by HPLC. Approximately 16 different molecular species were present in significant amounts with peak 10 (16:0,20:4), 11 (16:0, 18:2) 20 (18:0, 20:4) and 21 (18:0, 18:2) as the predominant ones. In response to EPO DG changed only in quantity and not in the presence or absence of specific species. Two important patterns were formed: one which peaked at 10 sec then decreased while the other peaked only at 40 min. Since the molecular species of phospholipids (PL) may change in response to EPO we determined the effects of EPO at 10 sec, 40 min, and 1 hr on each PL. Minor decreases occurred which corresponded to the increases in DG. Analysis of water-soluble headgroups was carried out by labeling PC and PE in medium which contained 5 μCi/ml [^{3}H]choline and [^{3}H] ethanolamine. There was an increase in choline release at 40 min and 1 hr. whereas ethanolamine was released as early as 10 sec, suggesting also an important role for ethanolamine for DG synthesis, in addition to PC. From the patterns of all molecular species, we could not find any relation between PA and PI. DG and molecular species were similar to some early peaks in PI. We conclude that detailed analyses of molecular species of phospholipids produced in response to EPO suggests two important temporal patterns.