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NON-EQUILIBRIUM BEHAVIOR OF SOME BRAIN ENZYME AND RECEPTOR SYSTEMS

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The previous article in this review dealing with the regulatory properties of brain tyrosine (TOH) and tryptophan hydroxylase (TPOH) systems (1) emphasized (*a*) *multidimensionality:* almost all the components present under physiological and assay conditions, ranging from electromagnetic fields through hydrophobic ligands to reducing equivalents, influence in one way or another the rate functions representing catalytic activities; (*b*) *nonlinearity:* critical zones of ligand concentration, curvilinear, even intermittent amount-effect functions, and inconsistencies among results depending on small differences in parameter values are consonant with the prominent role of cooperative interactions among the many stated and unstated dimensions (coordinates) regulating catalytic systems; (*c*) *conformational instability:* in the protein macromolecular components of TOH and TPOH, conformational instabilities are evidenced by 20 years of failure to purify the enzyme proteins in amounts useful for systematic extensive kinetic characterization, by their extreme friability under conditions of storage, by their multiplicity of reported molecular weights and kinetic constants, and by their markedly increased ease of denaturation and precipitation after the removal of components from their normal milieu by dialysis and enrichment procedures. That article expressed hope that a multivariate approach to studies of TOH and TPOH regulation could be developed to allow data reduction through pattern analysis in place of one- or two-variable kinetic experiments and quantification by Michaelis or Hill constants (1).

This review updates the 1978 one with respect to research on the regulatory properties of TOH and TPOH as reported in the literature through early 1983 with the exception of the cyclic nucleotide-protein phosphorylation schemata (recently involving calmodulin), the current status of which is reviewed else-

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where (2–8). The emphasis here will be on a quantitative approach to qualitative patterns in behavior of complex nonlinear systems like these, ones with which the molecular psychopharmacologist must continually deal. The principles derived will be extended to nonlinear phenomena seen in the currently popular ligand-binding systems with a few examples. The relevance of a new approach to cross-disciplinary pharmacological studies of brain function will also be demonstrated. A conjecture concerning a new structure-function approach to brain polypeptides emphasizing solvent-mediated dynamic macromolecular stability will serve to integrate these concepts.

The mathematical formalisms of this approach are properly derived from modern approaches to stochastic differential equations, including phase transition theory (8a–13) as enriched by current advances in nonlinear dynamics (14–20). This difficult and esoteric theoretical route is not practically useful to those of us working in laboratories of biochemical pharmacology. The emphasis therefore will be on geometric intuition (the behavior of functions in the phase plane), physical mechanistic images, quantitative indices derivable from ones familiar to those who have used elementary statistics, and a metaphorically representative equation that, despite the complexity of its behavior, is both easily understood and numerically solvable on a hand calculator. Elementary representations of solvent entropy, the hydrophobic effect, and macromolecular dynamics from current research in the physics of globular proteins in solution will be used to explain the *solvent-mediated allosteric principle* treated here as the prepotent influence of substrates, ligands, and drugs on the nonlinear kinematic behavior of brain enzymes and membrane receptors via alterations in their *dynamical stability*.

THE ALLOSTERIC PRINCIPLE: SOLVENT ENTROPY, THE HYDROPHOBIC EFFECT, AND MACROMOLECULAR STABILITY

Restraint of autonomous motion among 37° heat-perturbed water molecules, decrease in water degrees of freedom, can be caused by the reorganization of the previously random hydrogen-bond-preserving network of water around non-polar solutes (21, 22). This has been called the hydrophobic effect, bond, or interaction (23–25). The energetically significant negative entropy created by hydrogen-bonded water straddling hydrophobic moieties drives them together, configuring the behavior of biopolymers in solution (26, 27) and, along with the finer adjustments of internal and external hydrogen bonding, plays the major role in globular and membrane protein structural and dynamical stability (28–31). Charged hydrophobic solutes in aqueous solution, e.g. biogenic amine or polypeptide salts, reduce the heat capacity and entropy of the system due to both electrostatic influences and those related to hydrophobicity

(32). The intrinsically dynamically unstable viscoelastic globular protein in solution (33) is perturbed by heated solvent molecules into large, rare, autonomous "breathing" motions with time constants in minutes (34–38). The functional implications of this fluctuating protein admittance have been established by studies such as those demonstrating the need for macromolecular motion to make room for the trajectory of CO-to-protoheme and myoglobin internal binding domains (39–41). If the temporal-spatial randomness of the more frequent, small, fast, solvent molecule-driven macromolecular conformational fluctuations is reduced by charged hydrophobic ligands competing for solvent entropy and the protein's motions gather to become large and coherent, heat capacity calculations show that the molecules contain more than enough intrinsic energy (38 kcal mol^{-1} for a protein of molecular weight 25,000) to be driven through a trajectory of progressively less stable, more active states (folding intermediates) (42, 43), ending in denaturation (44). Long-known examples of such ligand-induced processes involve denaturation of protein via the reconfiguration of solvent dynamical structure by urea and guanidine salts (see Figure 12; 45, 46).

When a charged hydrophobic ligand is itself the concentration-dependent participant in the pattern of reaction rates used to characterize the regulation of brain enzymes (e.g. an aromatic amino acid, tetrahydrobiopterin cofactor [BH_4]) or membrane receptor binding (by a drug or polypeptide), the protein stability-dependent catalytic or binding behavior (41, 47) becomes an intrinsically complex nonlinear function of the changing reactant or ligand concentration $[R\text{-}L]_i$. We call this dual action of [R-L] the solvent-mediated allosteric principle; as is implicit in the case of t, the index i indicates its consideration in discrete steps over changing concentration. In tightly conserved water spaces like a test tube or the brain (48), all molecules influence the solvent-mediated behavior of all others in an almost infinite system of partial differential relations, which we dimensionally reduce in expression via the solvent entropy, a mediating quantity much like currency in a complex economic system. Such an arrangement represents a global dynamic system requiring statistical rather than deterministic characterization of its flow. Contrived experimental conditions can generate small parameter zones of linear behavior, indices called affinities, and an apparently deterministic kinetic system based on reduced sets of ordinary differential equations, but such approaches suppress the expression of most of the influential variables and the nonlinear phenomena that occur when systems are examined within realistic ranges of concentrations and ratios, particularly in aqueous solvent.

We should note that post-Boltzmann notions of entropy as explored in the context of modern mathematical research in ergodic theory with particular relevance to mixing indicate that between the limits of randomness and strict periodic order there are many, perhaps a practical infinity of, invariant meas-

ures reflecting informationally metastable states (14, 15, 49–55). This suggests that exquisitely specific, subtle, distributed brain codes can be built from conditions that have previously been regarded as electromagnetic and chemical randomness. For example, entropy as a distributed property of pharmacologically altered solvent structural dynamics might constitute the code for the 15–20 discriminable drug-state-dependencies of behavioral paradigms that influence all neurobiological functions (56). Systematically applied measures of metastable stochasticity may supply a cross-disciplinary language for the pharmacology of brain function as a global dynamical system (57, 58). As James Clerk Maxwell wrote, "The true logic of this world is the calculus of probabilities."

THE PHYSIOLOGICAL CONDITIONS AND BEHAVIOR OF TOH AND TPOH

Despite its 1:1 reaction stoichiometry (59), relative to its tyrosine (TYR) and tryptophan (TRP) cosubstrates BH_4 is in far-from-equilibrium concentrations in several regions of rat brain (60–66a). As low as 3–5 μM in regions active in biogenic amine biosynthesis such as rat caudate, compared with amino acid concentrations in the range of 15–40 μM, the cofactor is below the affinity constants of the mixed function oxygenases for it (67, 68), including the most recent estimates for purified TOH (220–394 μM) and TPOH (119 μM) (69–71). When the physiological catalytic ratios of 3:15 μM BH_4 to TYR are simulated in vitro in a crude caudate nuclear homogenate (72), dihydroxyphenylalanine (DOPA) synthesis rates range from 3–5 pmol/mg protein/minute. At 10:10 μM ratios of BH_4 to TRP, similar levels of 5-hydroxytryptophan product formation are observed in crude rat raphé nuclear homogenates (73). In vivo measures of rat caudate dopamine turnover (74, 75) show a rate of 30 nmol/gram tissue/hour, which converts to 0.5 pmol/mg tissue/minute, and with a rough estimate of brain weight as 10% Lowry protein, a rate very close to the in vitro catalytic velocities under conditions of physiological reactant ratios emerges: 5 pmol/mg protein/minute.

Steady-state kinetic studies of TOH require very high reactant concentrations to generate linear functions with small (gaussian) variances. They characteristically exploit BH_4 to TYR ratios ranging from 100:30–1100:15 (76–81). However, reaction-sequence studies conducted that way have limited physiological significance because of their order-of-magnitude distortions in reactant concentrations and ratios and the absence of control of the oxygen concentration parameter, TOH and TPOH being unsaturated at ambient levels (1). Recent studies have confirmed work (82) indicating that O_2 is a regulatory ligand as well as a cosubstrate, i.e. an $[R\text{-}L]_i$ (82a–87). A recent study combining pyrimidine cofactor analogues and heavy oxygen labeling to ex-

amine the reaction mechanism also suggests that a cofactor-oxygen adduct may be the first intermediate in the amino-acid hydroxylation process (88, 89), consistent with the earlier speculation that addition is partially ordered with respect to O_2 (1).

In vitro studies using a BH_4 to amino-acid ratio in the range of 2:1–1:1 tend to make more prominent the inverted U-shaped functions in the kinetics of both TOH (70, 79, 90) and TPOH (67, 71, 91), and similar evidence of this nonlinear behavior has been observed in catecholamine biosynthesis rates in vivo in response to graded loads of TYR (92). In vivo stoichiometry of reaction rates, but not the effect of BH_4 as $[R\text{-}L]_i$ on macromolecular stability, may be regulated by apparent rate-limiting levels of quinonoid dihydropterin reductase (QDPR) (93). In addition, the interactive TOH (TPOH)-QDPR shuttle along with a diffusive delay creates an opportunity for the biosynthetic oscillations of a metabolic reaction-diffusion system (94). In contrast, without competitive kinetics, inactivation of either TOH or TPOH by abnormal isomers of BH_4 in a concentration- and (of significance for macromolecular stability) temperature-dependent way reflects the hydrophobic ligand role of BH_4 concentration as an $[R\text{-}L]_i$, inducing activating-inactivating conformational transitions (95–99). A similar explanation can be invoked to account for the parabolic shape of uncompetitive DOPA-inhibition functions (81). When still farther from equilibrium, i.e. at more nearly physiological ratios of BH_4 to amino acid, 1:5 for TOH and 1:1 in the TPOH system, over small steps in $[R\text{-}L]_i$ or time t the kinetic velocity emerges as pharmacological ligand-sensitive, nonlinear, and bifurcating functions [called multiple saturation plateaus in earlier studies of regulatory enzymes (100–102)], integrals demonstrating discontinuous transitions among multiple stable states induced by the progressive increases in the solvent-mediated force term, $[R\text{-}L]_i$.

Examined over t, the same far-from-equilibrium, physiological conditions generated periodic, quasi-periodic, and non-periodic ("chaotic") oscillations characteristic of systems with multiple stability and resembling those seen in studies of the glycolytic and peroxidase-oxidase enzyme systems (103–108). This variety of behaviors over $[R\text{-}L]_i$ and t has been observed in brain TOH and TPOH systems (58, 72, 73, 94, 109–112). It appears that the high BH_4 levels and abnormal ratios of reactants used in the past in most studies of TOH and TPOH kinetics in vitro served to linearize catalytic dynamics over $[R\text{-}L]_i$ and t, suppressing a more complex and subtle chemical coding capacity intrinsic to multidimensional, nonlinear biogenic amine regulation. Patterns of dynamical behavior in TOH and TPOH are exquisitely sensitive to small changes in BH_4, but perhaps not stoichiometrically as much as to its influence as a charged hydrophobic ligand, an $[R\text{-}L]_i$ (72, 73), a conjecture supported by the finding that levels of pterin analogues that induce amphetamine-like hyperactivity and stereotypy altered the dynamics but not the mean catalytic velocity of TOH

(113). In this context it is relevant that in experiments using a coupled QDPR-phenylalanine hydroxylase assay for BH_4 at doses inducing behavioral stereotypy but not decreasing striatal dopamine synthesis (74, 75), L-amphetamine was unique among 35 psychotropic drugs examined in decreasing the pterin significantly (72, 114–116). That finding was confirmed recently using more specific high-performance liquid chromatographic (HPLC)-fluorescence detection (62; E. H. Y. Lee & A. J. Mandell, manuscript in preparation). It is perhaps as charged hydrophobic macromolecular stability ligands, $[R\text{-}L]_i$, that both amphetamine and amphetamine-induced changes in BH_4 dynamics (75, 117) alter the regulatory properties of TOH and TPOH (58, 118–125).

The physiological relevance of dynamic patterns in biogenic amine synthesis as seen in vitro with physiological reactant ratios is consistent with growing evidence in vivo of metastable statistical patterns of fluctuation in brain biogenic amine synthesis and nonlinear diffusion in baseline and perturbation-induced biogenic amine waves ("flying W's") revealed by electrochemical voltammetry (R. Adams, personal communication, 1983; 126–131). A time frame in minutes characterizes the in vitro biogenic amine catalytic statistical fluctuations (see above), the electrochemical relaxation waves of voltammetry, the $t^{1/2}$ of the early biogenic amine turnover studies (132–135), the relaxations of the largest motions of globular proteins in solution (34–37, 136–140), the average periods of the glycolytic and peroxidase oscillators (141, 142), and the pulsatile motions of brain cells in tissue culture (143). The relatively narrow range of mean mass of the monomers of globular protein enzymes (50,000–60,000) and their common solvent environment suggest a role for the statistical phasing of their instability-generated, time-dependent motions in sculpting the dynamic geometries of biological process. In this context the pharmacology of the regulatory properties of brain TOH and TPOH may implicate more general features of biochemical stability. In vitro, the use of low, physiological levels of reactants in realistic ratios acts as a noisy catalytic scattering system with non-gaussian behavior to statistically amplify these physicochemical instabilities. In vivo, as has been suggested with respect to the weak-field, extracellular electromagnetic wave processes in brain (144), the informational content of brain chemical processes may reside in these patterns of semi-ordered stochasticity (57, 58), a spatially distributed code generated by ion, solvent-macromolecular, and membrane interactions.

Statistical recurrence, a pattern of repeated zeros of a function, is an intrinsic feature of all bounded finite-dimensional stochastic differential systems (145); in Levy processes without finite higher moments, a characteristic equation representing its probability distribution (the Fourier transform into a distribution of wave numbers as in equilibrium systems), $f{:}P(x,t) \rightarrow dxP(x,t)e^{ikx}$, scales across the absolute dimensions of $[R\text{-}L]_i$ or t (11, 12). Thus, enzyme

behavior with near-periodic or aperiodic oscillating behavior in minutes, whose phase distribution is gathered by, for example, the regular perturbations of a light-dark cycle (146), can be expected to demonstrate more coarse-grained oscillations phased into diurnal rhythms in what has been called a self-similarity across scale (147). Such rhythms have been observed in brain and pineal TOH and TPOH (148–153). In the same vein, some protein motions manifest time scales of physical relaxation in months (136), and comparable seasonal rhythms in brain biogenic amine levels were the focus of a recent conference on biological rhythms in psychiatry (154).

Although the physical image of a protein fluctuating between metastable states is helpful, bounded multidetermined cooperative systems generate patterns of recurrence without such specific deterministic, cycle-generating mechanisms—all the participating components contribute to the emergent dynamic patterns. A coherent summation of the motions of the microdomains of a protein monomer can be visualized in this way (140, 155). Thus, TOH and TPOH product oscillations in minutes and seasonal variations in brain biogenic amine dynamics may reflect the same aggregate, scaling properties of a complex system.

Perhaps the simplest way to appreciate this phenomenon is in an examination of the wave forms generated by simple partial differential equation sets (156) and the characteristic scaling behavior of turbulent (dissipative) (157) and Hamiltonian (conservative) (52, 158) systems near zones of transition. Modern work in stability theory indicates that whereas small perturbations generate bifurcation (branching of the solutions of nonlinear equations) in fragile periodic dynamics, patterns of aperiodic recurrence are both sensitive and remarkably stable structurally (159, 159a). Temporal and spatial slippage in a cycle gives it the flexibility necessary to survive, although the biologist is often likely to regard this less regular geometry as meaningless noise. In systems similar to these biochemical and physiological systems, inability to predict behavior precisely using specifiable coefficients in differential equations led mathematicians to call such aperiodic oscillation chaos. A probabilistic approach to these chaotic dynamical systems, however, has shown them to contain invariant measure (160, 161).

THE BEHAVIOR OF TOH AND TPOH AS A MULTIDETERMINED $[R\text{-}L]_i$ and t-DEPENDENT FLOW OF PROBABILITY

Consistent with the findings of the previous review (1), there is continuing evidence that TOH and TPOH are sensitive to a large array of physiologically relevant influences. The regulatory importance of the dynamical physical state of rat caudate TOH (162), emphasized in this development and studied pre-

viously in relation to membrane and membrane-like components (78, 163–166), appears to be supported by recent confirmation of the role of particulate versus soluble subcellular location in determining the affinity of the enzyme for BH_4 but not for TYR (167); by current ultrastructural immunocytochemical studies demonstrating that 82% of TOH is in membrane-specialized punctate varicosities, TOH-relevant microscopic structures reported for the first time (168); by incubation with bacterial phospholipases altering its kinetic constants (169); and by a demonstration of both kinetic activation and inactivation (the characteristic multiphasic influence of an $[R\text{-}L]_i$; see below) by phosphatidylinositol (170). Phospholipid-induced, pH-dependent activation of rat brainstem TPOH has also been reported (171). The charged nature of native brain TOH and TPOH has been studied recently using a new mini-column isoelectric-focusing pH-gradient technique (J. H. Jackson & A. J. Mandell, manuscript in preparation) and is consistent with significant macromolecular and membrane interactions, kinetic changes in interaction with charged tubulin molecules (172), and the need to prepare an HPLC column with albumin to allow TOH recovery (173). The recent failure to relate activity state and adherence to membranes of adrenal TOH using histochemical staining is not surprising in that these membrane-depolarization, ion-sensitive phenomena (166, 174) behave like dynamical and not permanent histological changes (175). The sensitivity of both TOH and TPOH to negative electrostatic fields as first demonstrated for TOH in 1972 (78, 163) has been elegantly confirmed using heparin in interactional studies with polypeptides (176). Some of the same effects of chondroitin sulfate polyelectrolytes have been reported for TPOH (177, 178). The field-like sensitivity of TOH and TPOH to the influence of ions (179) and the electromagnetico-chemical environment in which brain TOH and TPOH function suggest that the regulatory effect of anions, including carboxylic acids (172, 180) and electrical field stimulation (181), may not be unrelated. The Gibbs-Donnan counterpoint to electrical-chemical negativity in neuronal membrane dynamics, the cations including H^+, K^+, Na^+, Ca^{++}, Mn^{++}, Mg^{++}, the actions of chelating agents, organic cations, iontophores, K^+ active cardiac glycosides (and other polyhydroxy compounds, including ascorbic acid and glucose) have also been shown to play influential roles in the regulation of these brain enzymes' conformational-kinetic stability (181a–191). Related to the issue of pH is the role of specificity of the reducing conditions of the enzymatic reaction, including sulfhydryl groups, protection against H_2O_2, and the role of iron, which has not yet been irrefutably demonstrated to be at the enzymes' reaction center (71, 82a, 88, 192–197).

Beyond the long history of both the activation and inactivation properties of BH_4, TYR, and TRP (1), of greatest relevance to the $[R\text{-}L]_i$-TOH (TPOH)-solvent interaction with respect to the induction of a destabilizing-activating-denaturing trajectory for the physical change in the enzyme protein is the recent and remarkable report of Kaufman & Mason (198) indicating that hydrophobic

amino acids like methionine and norleucine activated the hepatic mixed-function oxygenase phenylalanine hydroxylase with respect to its physiological substrate. In addition, and consistent with the activating induction of large coherent unfolding motions by hydrophobically constrained solvent and resulting increased ease of substrate approach to the buried active site (39–41, 47), the structural requirements of amino-acid substrates were relaxed (both methionine and norleucine were hydroxylated) when the enzyme was activated in any of several different ways. The same solvent-mediated dynamical stability factors may account for uncompetitive influences on TOH by other non-specific hydrophobic moieties such as unphysiological pterins (96–98) and the tetrahydroisoquinolines (199). There have been several demonstrations of the anatomical proximity of a variety of biologically active peptides considered here to be charged hydrophobic ligands for TOH and TPOH (200–204), as well as catalytic activation by some (205–207), including chains as large as albumin (208), which also stabilizes (173). In vivo evidence of enzyme inactivation by large loads of tyrosine (92), and even the mysterious antidepressant efficacy of D-phenylalanine, equivalent to that of a combination of the D and L isomers and manifesting a two-week latency as is required for the antidepressant effects of tricyclic drugs, small doses of phenothiazines, or tryptophan loads (209, 210), may be explained by a charge and hydrophobic-effect increase in brain-solvent free energy and the induction of an associated destabilization-activation conformational trajectory of TOH and TPOH associated with an antidepressant effect-correlated increase in brain biogenic amine synthesis (211).

There is rather clear evidence that a multiplicity of ligands influential on TOH and TPOH is always present in vitro, that in the brain these systems manifest multiple quasistable states, and that physiological reactant concentrations and ratios generate non-equilibrium catalytic scattering behavior rather than gaussian linear or curvilinear functions manifesting only up-and-down regulation. These conditions, then, bring to brain chemical processes the potential for expression as subtle and complex as the behavioral output of the brain itself. We will proceed now to describe kinematic processes as changes in sizes and shapes in the geometries of the flows of probability, which can be quantitated and predicted by suitable equations, and portray both individual and phase-dependent molecular mechanisms as statistically defined patterns of global dynamical behavior. In these considerations the primary unit of data will be the A value:

$$A = \frac{\mathrm{E}x - x_i}{\overline{\mathrm{E}x + \dot{x}}} \qquad \text{[Eq. 1]}$$

the value of catalytic velocity, x_i, at a particular value of $[\text{R-L}]_i$ or t as a difference from expectation, $\mathrm{E}x$, the value of the corresponding point on a statistically determined regression line representing the aggregate of the data as

normalized by the sum of the average level, $\overline{Ex}$, and slope $\dot{x}$. This condition allows a function of increasing or decreasing velocity over $[R\text{-}L]_i$ or t, where t refers to $(dA/dt)_{[R\text{-}L]}$, to be treated as a normalized series of values over a zero slope. The dynamical behavior of the system is represented by a first-order equation in A:

$$\frac{dA}{d([R\text{-}L]_i, t)} = V(A;[R\text{-}L]_i, t) \qquad \text{[Eq. 2]}$$

where V is a velocity function of A. We condense this high-dimensional process into a first-order autonomous equation representing the phase velocity of A, eliminating its explicit dependence on $[R\text{-}L]_i$ or t:

$$\frac{dA}{d(H^+, K^+, Na^+, CA^{++} \ldots [R\text{-}L]_i \ldots t)} = V(A) \qquad \text{[Eq. 3]}$$

and

$$\int [R\text{-}L]_0 - [R\text{-}L]_i,\ t_0 - t_i \quad = \int_{A_0}^{A_i} \frac{dA}{V(A)} \qquad \text{[Eq. 4]}$$

Thermal inactivation studies of TOH and TPOH systems evidence three interconvertible kinetic conformations and a dynamical trajectory between them (99, 212–215), with normalized velocity levels of approximately $1.0 \rightarrow 2.5 \rightarrow 0.3$, suggesting an exponential relationship among the states. Conditions that facilitate activation also inactivate (1, 99, 169, 216, 217). Kept at room temperature and sampled every minute, rat raphé TPOH activity as a sequence of A values demonstrates this kinetic-conformational trajectory in one continuous experiment (Figure 1; 73, 218). Purified mouse mastocytoma TPOH also manifests an iron-reducing system (H_2O_2?)-sensitive set of three discrete states (219); those studies demonstrate normalized activity ratios of $1.0 \rightarrow 5.0 \rightarrow 0.2$, also suggesting three logarithmically (power law) related activity levels. Assuming a greater than root mean square proportionality under far-from-equilibrium conditions between the catalytic velocities and the average amplitude of the variations, RMS_A, we can represent the changes from activated (a), low activity (l), and baseline (b) states as a birth and death trajectory of A in the phase plane portraying the flow of probability as a one-dimensional dynamical system with a random distribution of phase (Figure 2). Described as the nonlinear spring-like folding-unfolding dynamics of globular proteins in solution with intermediate metastable states (42, 43, 220, 221), the viscoelastic protein (33) with reaction component-sensitive stability properties accrues a nonlinear macroscopic response to heated solvent perturbations over time,

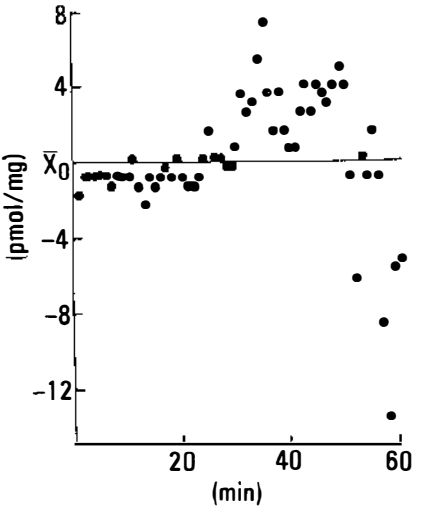

Figure 1

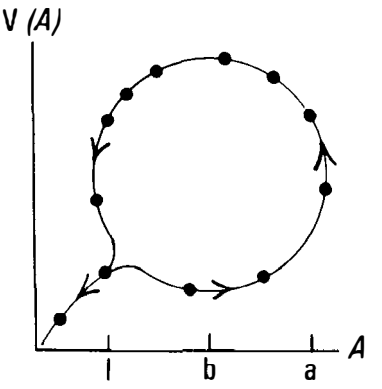

Figure 2

requiring a memory kernel, for example, an exponential instantaneous distribution of states, $G_\tau(b \rightarrow a)$ and $G_\tau(a \rightarrow l)$. As seen in the substrate activation-inactivation functions below, conditions that facilitate activation also augment the inactivation process rather symmetrically (216, 217), as in Figure 3, so that $G_\tau(b,a,l)$ can be represented by a convolution of exponential processes ($A_0 \exp^{kA}$, $A_{max} \exp^{-kA}$), which reconfigures the phase portrait of $A/V(A)$ into a hysteresis loop with singularities at the $dV(A) = 0$ transitions (Figure 4, left), seen perhaps more clearly in a potential energy graph of $A/U(A)$ (Figure 4, right) representing transitions through metastable states. Synchronization of phase among these globular protein enzymes with nonlinear oscillations of their cosubstrate admittances occurs in the regions of the singularities, $dV(A) = 0$

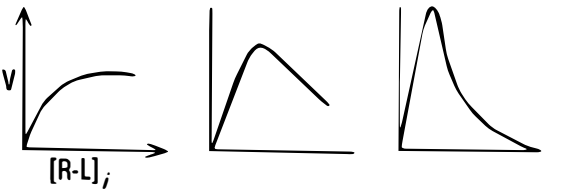

Figure 3

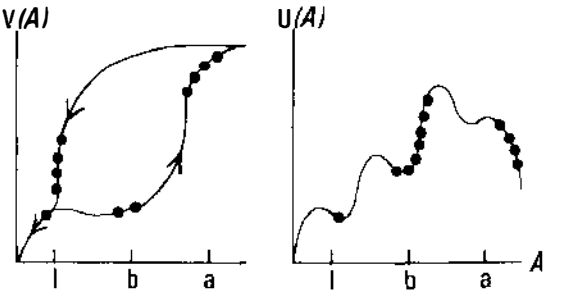

Figure 4

(146). In addition, these zones of transition, degenerate neighborhoods of multivalued inverses, f:V(A) → A, are the ones showing greatest changes in dynamical behavior with small changes in parameter values. Autonomously emergent changes as in Figure 1 suggest that physiological function and its regulation with respect to changes in chemical information flow require very little energy in addition to solvent perturbation in zones of molecular instability and associated changes in the distributions of phase. The trivial amount of energy required to regulate processes through their instabilities suggests a neurochemical explanation for the thermodynamically paradoxical findings that wild psychosis and sleep manifest the same mean levels of brain glucose and oxygen utilization in man (222, 223).

Periodicity (one frequency), quasi-periodicity (two frequencies), and aperiodicity (three or more distinct frequencies and/or chaos) have been observed in the product concentration fluctuations of TPOH (73, 111) and TOH over t as in Figure 5 (58, 72). Transitions between multiple dynamical regimes (see the protein denaturation curves in Figure 12) have also been observed across [R-L]$_i$ for TPOH (Ca^{++}) (Figure 6, left) (109) and TOH (TYR) (Figure 6, right) (72).

HOW MACROSCOPIC DYNAMICAL COMPLEXITY CAN EMERGE FROM ACTIVATION-DEACTIVATION PROCESSES IN A POPULATION OF ENZYME PROTEINS

The way in which parabolic manifolds portraying density-dependent processes, like those seen in the substrate kinetics of TOH and TPOH (Figure 3) generate

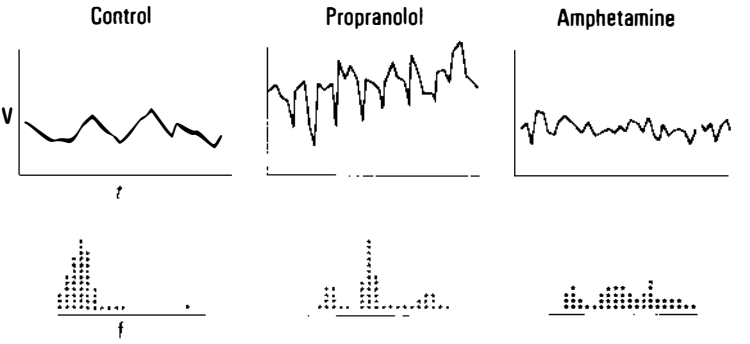

Figure 5

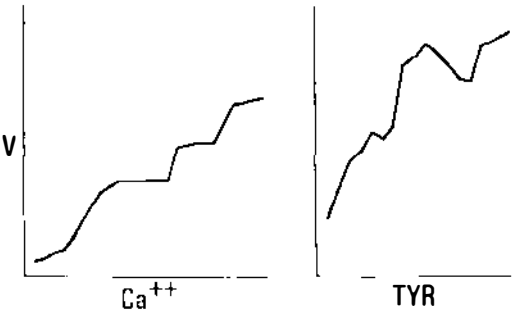

Figure 6

both periodic and aperiodic dynamics across small changes in parameter values is a current focus of interest in statistical physics (17–20). In the interest of a simplification not ordinarily permitted for a far-from-equilibrium system, we linearize G(τ) so that $d_A(\text{b} \rightarrow \text{a})/dt = \text{B}(A)$ and $d_A(\text{a} \rightarrow \text{l})/dt = \text{D}(A)$. The equation of motion for A then becomes:

$$\frac{d(A)}{d([\text{R-L}]_i, t)} = \text{V}[\text{B}(A) - \text{D}(A)] \qquad \text{[Eq. 5]}$$

At low values of $[\text{R-L}]_i$ or t, B > D, and beyond some critical transition D > B, as in Figure 3. A_0 can be seen as a stable fixed point and the second singularity, a metastable stationary state, is at $A_{\max}$, $d(A) = 0$, V[B(A)] = V[D(A)] as in Figures 3 and 4. It has long been known that increased density of active forms of TOH and TPOH produced by activation, dialysis, or steps toward purification leads to monomeric aggregation, loss of catalytic activity, and precipitation of denatured protein (67, 70, 71, 78, 90, 163, 170, 173, 196, 224–230). The mechanism may involve resonance in large, slow protein motions facilitating coherent, high-amplitude oscillations as in Figure 1 and progression

through three metastable states toward irreversible unfolding (44, 231). Thus, two kinds of inactivation are seen: D as a consequence of the trajectory through activation (as in Figures 2–4) and $\tilde{D}$, dependent on the presence of other activated monomers, i.e. $d(A) = A(B - D) - \tilde{D}A^2$. We combine D and $\tilde{D}$ in a single expression representing the density dependence of the inactivation process:

$$d(A) = V(BA - DA^2) \qquad \text{[Eq. 6]}$$

From the symmetries seen in Figure 3, $B(A) = D(A)$, $B = D = r$, a generalized force term that can represent [R-L], making the manifold:

$$d(A) = V[rA(1 - A)] \qquad \text{[Eq. 7]}$$

which in the context of the sequence of repeated samplings of TOH and TPOH over discrete steps of $[R\text{-}L]_i$ or t is the classical logistics map (232).

$$A_{t+1} = rA_t(1 - A_t) \qquad \text{[Eq. 8]}$$

This simple discrete difference equation generates a parabolic curve (Figure 7) whose slope is dependent on $[R\text{-}L]_i$ and whose evolutionary behavior over time resembles that seen in Figure 5.

More detailed development of stoachastic birth and death processes (12) shows them to generate bifurcations that reflect kinematic multistability, seen in Figure 6 and modeled by Equation 8 as in Figure 7. The oxidase-peroxidase system has long been known to display parameter-sensitive bifurcations from equilibrium to single and multiple frequencies and/or chaos (106, 142, 233–236). When periodic versus aperiodic (chaotic) behavior of Equation 8 is plotted as a function of r above values of 3.4 (237, 238), a pattern resembling that in Figure 8 is observed:

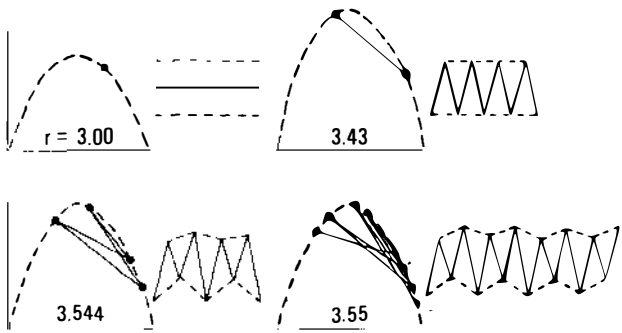

Figure 7

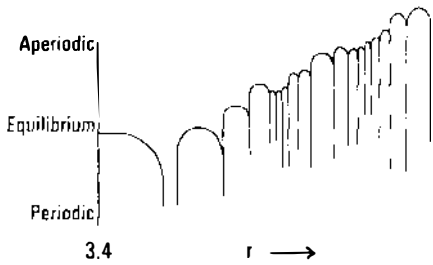

Figure 8

Chaotic regimes and those that are strictly or partially ordered in time are seen closely juxtaposed in parameter space (239). As operative across $[R\text{-}L]_i$, in addition to the feature of sensitivity to differences in initial conditions (161), this principle accounts for the instability manifested by the TOH and TPOH systems when examined under physiological, far-from-equilibrium conditions (72, 73, 111–112). Concentration-dependent stabilization and destabilization by BH_4 (98, 213), activation and inactivation by phospholipids (170), amino-acid substrate activation and inhibition (216, 217), multiphasic effects of increasing levels of in vitro amphetamine (58), and many of the conflicting reports of the influence of various ligands on these systems (1) are examples of these dynamics. The characteristic anomalous and wide clinical dose-response curves of psychotropic drugs, for example the dosage windows for the clinical efficacy of tricyclic antidepressant drugs, are consonant with the nonlinear stability properties of these biogenic amine biosynthetic systems over increasing $[R\text{-}L]_i$ (240, 241).

A STATISTICAL KINEMATICS OF NON-EQUILIBRIUM STEADY STATES: GENERALIZATION ACROSS NEUROPSYCHOBIOLOGICAL LEVELS

Most transform techniques useful in dealing with nonlinear systems (242, 243) are limited by the rather strict requirements of their mathematical assumptions. For example, the stationarity, convergence, and adequate sample length assumed by Fourier transform techniques, displayed qualitatively in Figure 5, are not fulfilled by 100-point studies in triplicate (72) of far-from-equilibrium enzyme system fluctuations (244–246). The third and fourth moments of the probability density distributions reflecting rare, high-amplitude events as seen in computer simulations of protein motion (140)—the critical fluctuations that bifurcate distribution functions (13)—require sample lengths beyond those now possible in brain enzyme kinetic experiments (247). In their place is sought a reliable and meaningful quantitative measure of the pattern of behavior of the A values across $[R\text{-}L]_i$ and t that would reflect the shape of the probability distribution, indicate the frequency content of the A value varia-

tions, portray the system's stability along the vertical dimension of Figure 8, reflect the number of independent phases or enzyme forms contributing to the process as its dynamic dimensionality, and scale across a wide range of intervals in time so that drug influences could be compared among several neuropsychobiological data bases. In combination with the RMS_A, the fractional characteristic exponent D_A, the geometric dimensionality of the A value integral (11, 147, 239, 248) serves these purposes quite well (72, 73, 94, 247). This power law dependence of measures made on cooperative biological systems is analogous to the scaling law descriptions of statistical physics (12). Repeated measurements of the catalytic activity of an enzyme homogenate over $[R\text{-}L]_i$ or t, synchronized by continuous rhythmic perturbation in a metabolic shaker (72, 73, 249), are transformed as in Equation 1, $f{:}V_i \rightarrow A_i$, creating the new, normalized series of A values upon which a measure of the texture, D_A, can be made with values ranging from 1 for a smooth line to 2 for an irregular, space-filling (two-dimensional) function.

The relationship between the roughness of the surface of a multidimensional volume representing a dynamic system and its underlying cooperativity as dimensionality (the number of independent coordinates projecting information onto the one-dimensional sequence of A values) can be analogized from the following argument (250). Removing the middle 90% of a line of unit length (dim = 1) leaves 10% at the "surface" of the two ends; removing a circle of diameter 0.9 from the unit disc (dim = 2) leaves about 20% at the surface; in dim = 3 the removal of a concentric ball of diameter 0.9 from the unit sphere leaves about 28% at the surface. In the limit the internal volume of a geometric object of diameter 0.9 and dimension ϕ, $(0.9)^{\phi} \rightarrow 0$ as $\phi \rightarrow \infty$. In the geometry of multidimensional volumes, the more independent contributors of mechanism or phase, the higher the dimensionality and the greater the arc length of the perimeter relative to its volume. The minimum number of unit balls of diameter ϵ, $N(\epsilon)$, required to cover the function increases with an increase in dimensionality (251), which is seen as an increase in D_A.

D_A is calculated as the slope created when the log of the diameters of a sequence of increasingly larger spheres is plotted on the x-axis against the log of the number of balls of each size required to cover the function projected onto the y-axis. The more irregular the surface, the more crevices are lost by the progressively larger spheres, the steeper the slope, the larger the D_A (72). A microcomputer program for the calculation is available upon request.

With few mathematical assumptions and remarkable statistical stability, the geometric dimension has been applied successfully to electron spin relaxation measurements on myoglobin and ferricytochrome C (252). It can also quantify precisely the elusive behavior of far-from-equilibrium kinematic scattering systems. It serves as the characteristic exponent of non-gaussian distribution functions that are without finite higher moments (11, 253); it describes the

shape of the tail of these distributions as an extremal measure that scales as the mean first-passage time (254); it gives a single numerical value to long-range correlations, thus serving as an index of the frequency content of the process (255); it serves as a numerical solution to undifferentiable functions (256); it transforms directly to a measure of the vertical dimension of Figure 8 called the sum of the Lyapounov exponents, quantifying the system's stability (239, 257–259). Since D_A represents the convergence in a relationship between a measure and its measurement, it has symmetry with respect to dilation, i.e. the index is independent of its absolute size. In this way, D_A is self-similar across temporal scales in processes like the internal symmetries of eddies within eddies within eddies in the dissipative dynamics of hydrodynamic turbulence (260, 261), the infinity within conservative Hamiltonian systems in the alternating patterns of invariant curves, and stochasticity seen in the homoclinic regions between attractor domains (14, 15, 52, 158, 262, 263). With the enzymes of the biogenic amine systems omnipresent in brain regions, it is perhaps not surprising that the effects of ligands such as amphetamine, lithium, chlorimipramine, and thyrotropin-releasing hormone (TRH) demonstrate similar alterations in D_A and D_A-like dynamics in TOH and TPOH systems, [^{3}H]-spiroperidol binding, interspike intervals of single units, electroencephalographic dynamics, animal behavior, and clinical response (58, 94, 263a–266). A similarity in the power-law dependence of multiple measures made on a single complex system is consistent with its status as an integrated organization (267), not an unreasonable claim with respect to psychotropic drug-influenced central nervous system function.

The way the D_A to RMS_A relationship as $\delta D_A/\delta RMS_A$ reflects the system's cooperativity as examined over an *ensemble* of experiments under the same conditions can be seen in the two contrasting views of how fluctuations in complex nonlinear systems evolve over time (Figure 9).

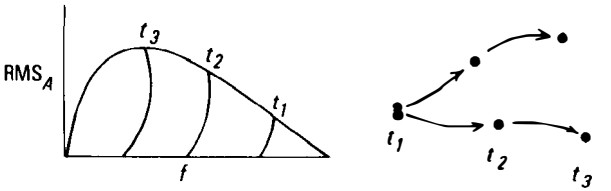

Figure 9

The Eulerian view (Figure 9, left) shows that fluctuations enter the small scales of motion as fast, frequent perturbations by heated solvent molecules and propagate across the 16 time scales of protein motion from 10^{-12} seconds (268) to minutes (136), the total error energy amplitude represented as the RMS_A (269). For globular proteins in solution this process is influenced by changes in solvent ΔG induced by charged and hydrophobic ligands. The Lagrangian view

(Figure 9, right) describes the extent of the maintenance of the neighborhood topology in the evolutionary process, a systems property called mixing reflected in the value of D_A (52, 161); we see a high mixing system in which two points that were together initially become widely separated over time. This effect is regulated by ions, drugs, and other influences on phase that promote or prevent the synchronization of molecular motions. The differential of the D_A to the RMS_A, a dispersion relation shown in Figure 10,

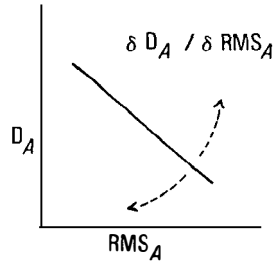

Figure 10

reflects the degree of coupling, the maintenance of the integrity of a neighborhood of values over time, in what can very generally be called a stochastic frequency-amplitude graph. A negative slope reflects a cooperative dynamic that is more subject to bifurcations and phase transitions, whereas a zero to positive slope indicates relative independence among the contributing elements or phases and the greater stability of "noisy periodicity" (94, 159a, 270). The differences between the slopes of the regressions of ensembles from experiments conducted under various psychopharmacological conditions can be tested for statistical significance (73, 230, 247).

Psychotropic drug-induced increases in $-\delta D_A/\delta RMS_A$, as seen at the molecular dynamic level in the TPOH system in the presence of tricyclic antidepressant drugs, are associated with hyperbolic, bifurcating saturation functions and the emergent periodicity and phase transitions characteristic of systems of anharmonic oscillators perturbed by increased coupling (271) in several neurobiological and clinical phenomena (73, 263a, 265, 266, 272). Decreases in $-\delta D_A/\delta RMS_A$ induced by physiological levels of lithium are associated with more sigmoid saturation functions and demonstrate the stability of systems composed of more independent elements (12, 73, 265, 266, 272). Depending upon dose, amphetamine induces both these contrasting conditions in a variety of neuropsychobiological contexts (58, 94, 263a, 273).

NON-EQUILIBRIUM BEHAVIOR AS MULTIPLE RECEPTOR LIGAND-BINDING PROCESSES

The influence of a ligand on the kinetics of its own binding behavior as an $[R\text{-}L]_i$, the allosteric principle (274), was invoked long ago to explain the

nonlinear behavior of oxygen binding to hemoglobin (275–277). Those classical non-Langmuir, non-Michaelis functions manifested fractional characteristic exponents of about 2.8 (278). Such behavior is easily analogized to more modern views of the nonlinear dynamics of ligand-induced conformational changes in macromolecular and membrane stability via ligand-induced changes in solvent entropy (26–28, 30, 31, 35–37, 279) called the hydrophobic effect (23–25). One recent demonstration of the role of solvent influences on macromolecular motion as reflected in receptor binding kinetics was a direct one exploiting systematic variations in solvent viscosity (41).

Ligand-binding techniques used in current pharmacological studies exploit extremely high concentrations of (cold) hydrophobic ligands, most of which generate multiple discontinuities in the saturation functions (280, 281), not unlike the bifurcational behavior seen in Figure 6 over $[R\text{-}L]_i$ and in Figure 5 over t and modeled by Equation 8 and Figure 7 (see also Figure 12). In this context, binding is viewed as adherence to a macromolecular-membrane moiety conformationally altered in a nonlinear manner in the direction of denaturation and precipitation by increasing concentrations of charged and hydrophobic ligands. These dynamics are consistent with a degree of structural-dynamical specificity of the ligand as well as the less specific nonlinear force characteristics of $[R\text{-}L]_i$ as seen in Figures 7, 8, and 12. Anomalous behavior in time observed in the early pharmacological ligand-binding studies (282), i.e. the demonstration that the low-affinity system saturated several minutes before the high-affinity one, suggests conformational interconversion as in Figures 1, 2, and 4 rather than the simultaneous presence of multiple receptor membrane proteins. An extensive new literature on specific coding in entropies (see above) makes structural specificity transformable into equal or even more specific solvent-mediated dynamical messages and offers an explanation for the ever growing receptor-system kinetic heterogeneities and inconsistencies in the experimental literature (282a, 282b). Bathing a system of relatively homogeneous nicotinic-cholinergic microsacs from *E. electricus* and *T. marmorata* in high concentrations of cholinergic ligand is the condition under which the depolarization mechanism is desensitized, the time dynamics bifurcate into fast and slow processes (283), and multiple kinetic binding functions can be observed (283–287). Examples of $[R\text{-}L]_i$-induced bifurcations in kinetic functions are seen in Figure 11: on the left in $[^3H]$-TRH binding to pituitary cell membrane (288); in the center in $[^3H]$-etorphine binding to liposomes containing cerebrosides (289), a preparation not inconsistent with the orderly and complex kinetics of binding to other non-biological, surface-active materials (290, 291); and on the right in a nonlinear Scatchard plot the use of which is actually inappropriate for nonlinear systems (292), with two high-affinity unstable stationary states in $[^3H]$-spiperone-haloperidol competition binding to an olefactory tubercle crude membrane preparation from the mouse (293).

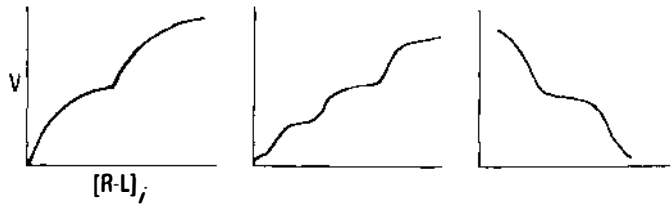

Figure 11

The similarity of these iterative binding functions to classical multiphasic protein denaturation curves over increasing concentrations of solvent-active, charged hydrophobic ligands such as urea or guanidine salts (45) and lithium bromide (294) is rather striking (Figure 12).

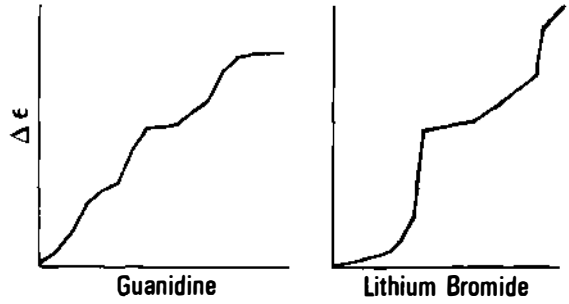

Figure 12

Multiple stable states across increasing concentrations of charged hydrophobic [R-L]$_i$ as in Figure 11 generate the expected instabilities in the time domain as in Figure 13: [^{3}H]-cAMP binding to purified plasma membranes from *D. discoideum* (295) on the left; cumulative [^{3}H]-spiroperidol binding to crude rat striatal membranes (296) in the center; and a similar preparation with more frequent sampling displayed as differences from mean velocity (264) on the right.

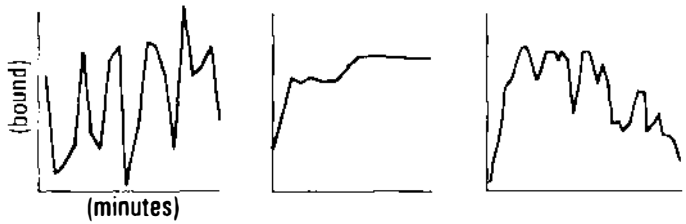

Figure 13

Most of these complex nonlinear behaviors, called surface phenomena in the context of [^{3}H]-spiperone binding to crude striatal membranes (297), were

demonstrated several years ago in the context of studies of insulin-receptor interactions (297a, 298) and included the multiphasic, concave upward Eadie-Scatchard plot (299); the same $[R\text{-}L]_i$-induced increases and decreases in binding as seen in Figure 3 and modeled in Equation 8 and Figure 7 (300, 301); and anomalous time-dependent dissociation behavior in the context of the affinities of ligand binding saturation functions (297a, 301, 302). A dynamic (Figure 14, right) in contrast to a structural (Figure 14, left) scheme portraying the interactions between $[R\text{-}L]_i$ and membrane receptors is seen as an exchange of solvent entropies (303) between ligand and receptor polypeptide chains (279), a system with, if anything, more degrees of freedom with respect to the specific encoding of information than that of a static, lock-and-key structure. A successful ligand-membrane receptor interaction may depend upon resonance in the ligand-induced, solvent-mediated receptor response function.

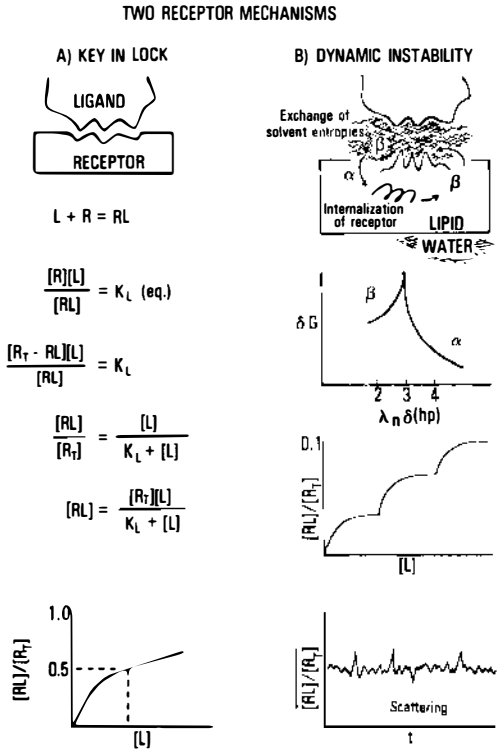

Figure 14

The receptor peptide is portrayed as a transitional β-strand-like form, unstable in water (29, 304–307) and a configuration seen often in binding domains of

proteins (308–311). It is perturbed by a $[R\text{-}L]_i$-induced change in the neighboring solvent structural dynamics into a volume-reduced, more α-helical form as seen in lysine and leucine copolymer transitions (314), and hydrophobic negative solvent entropy drives it into the lipid bilayer, a process called receptor internalization (312). In this less hydrophobic, lower ΔG environment it can reform. Similar solvent-mediated peptide-peptide dynamics are observed in studies of protein folding (306). It should be noted that there is evidence that the internalization process in non-central nervous system tissue is associated with receptor-mediated endocytosis (313, 314). The membrane perturbation associated with these events could serve as a low-energy, instability-induced trigger for the subsequent transconductional events. Exquisitely solvent structure-sensitive rates of spontaneous depolarization (for example, after small changes in sodium concentration) characterize the behavior of artificial lipid bilayer models of neuronal membranes (315; M. Montal, personal communication, 1981).

Since water structure and dynamics in a closed system are temporally and spatially distributed properties (21, 22), global properties of pharmacological and peptide charged hydrophobic ligands can be rationalized as induced changes in solvent entropy over large regions of the brain and reflected in influences on macromolecular and membrane stability. This suggests that predictions about the relationship between structures and functions of families of $[R\text{-}L]_i$'s could be predicated on the basis of their influences on solvent entropies. Due to the precise quantification of this property in kcal for each amino acid, brain polypeptide structure and function can serve as a test of this $[R\text{-}L]_i$, solvent entropy hypothesis.

A SOLVENT ENTROPY SEQUENCE APPROACH TO BRAIN POLYPEPTIDE STRUCTURE AND FUNCTION

Recent systematic studies of codon substitution errors and secondary and tertiary structural equivalences in the evolution of polypeptide chains indicate that amino-acid exchanges are made on the basis of similarities in their affinities for water (309, 316, 317). Four families of five amino acids each have been characterized by conversion as energies via their equilibrium kinetics of transport from organic solvents to water as an index of hydrophobicity in kcal/mol: 0.00 → 0.10, 0.66 → 0.87, 1.57 → 2.17, and 2.67 → 3.77 (318–320). In β short spans, consecutive amino acids alternate between low and high values for hydrophobicity; a hydrophobic side chain is surrounded by two hydrophilic or apolar residues (321–323). α-Helical short spans have a two-fold greater wavelength in the hydrophobicity sequence of their residues in which on the average two hydrophobic side chains are followed by two that are hydrophilic or apolar (323–325). The increased adjacency of hydrophobic

groups in a longer wavelength, α-helix-like structure leads to more negative solvent entropy-forced self-aggregation between the residues (a critical mass may serve to recruit even more of the chain), a reduced volume of solvent occupancy, and less solvent structural distortion; a sequence varying more frequently between hydrophobic and hydrophilic or apolar residues as in a β-strand occupies a greater solvent volume and induces greater destabilizing ΔG in solvent entropy (307). For example, α-helices become conformationally stable in solution in 10^{-7} seconds (326), whereas the β-conformation requires minutes (327, 328).

On the basis of these findings and the above development involving solvent-mediated macromolecular stability, two members with well-established differences in potency were selected from each of six families of neurobiologically active peptides. The polypeptides were normalized to equivalent lengths; the sequence of deviations from mean hydrophobicity in kcal was determined for each peptide, treated as in Equation 1, and its D_A value calculated (303, 329). As $[R\text{-}L]_i$, the faster-frequency, more β-strand-like series, having a higher D_A than the more smoothly varying α-helix sequences, were predicted to generate higher solvent ΔG-mediated macromolecular and membrane instability, with a resulting increase in central nervous system potency (Figure 15).

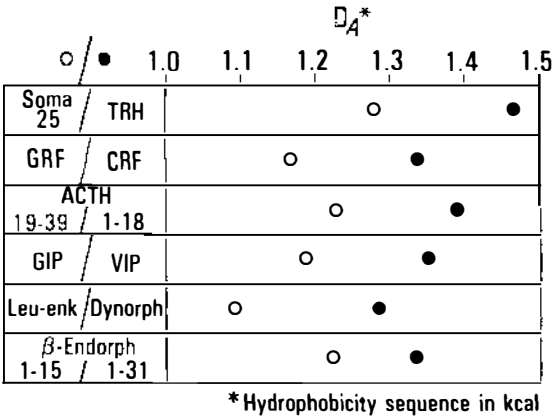

Figure 15

The chart demonstrates that a higher D_A (solid dot) is manifested by the more behaviorally activating of each pair of peptides, i.e. by thyrotropin-releasing hormone than by somatostatin-25, by corticotropin-releasing factor than by growth-hormone releasing factor, by the first segment of adrenocorticotrophic hormone than by the second, by vasoactive intestinal peptide than by gastrointestinal peptide, by dynorphin than by leu-enkephalin, and by the entire β-endorphin than by the first half of its sequence (330–336; R. Guillemin,

personal communication, 1983; P. Brazeau, personal communication, 1983). The nonlinearity of influences of an $[R\text{-}L]_i$ on the activity and stability properties of TOH and TPOH, seen in Figures 3 and 6 as modeled in Equation 8 and Figures 7 and 8, is also observed in the excitatory, inhibitory, and nil effects of the same peptide, depending on neural cell type, anatomical location, and associated neurotransmitter ligands (335, 336). The characteristic partial antagonisms among the participants in a multidetermined system rather than the monotonic ordering of values of the geometric dimension on the hydrophobicity sequence in relation to effect, as in Figure 15, may better predict the actions of related neural peptide pairs. For example, substance P ($D_A = 1.10$), dense in terminals A_{10} mesencephalic dopamine cell bodies, induces an amphetamine-like hyperactivity syndrome when infused into the ventral tegmental region (337); neurotensin ($D_A = 1.34$), located similarly (338), blocks amphetamine-induced hyperactivity and stereotypy when given intracerebrally (339). Perhaps an aggregate of regionally involved brain peptides can be summed logarithmically like Lyapounov exponents of stability (237–239) in order to predict their multiplicatively summed influence on a system (303).

This approach also suggests the possibility that the one ligand molecule-one receptor protein moiety stoichiometry implicit in the use of molarity instead of weight as the meaningful unit in studies of dose-response functions of polypeptides may not be correct. For example, the difference in the exponents, D_A, to the base 2.5, the ratio of the masses per molecule of dynorphin versus leu-enkephalin as the log of the dose equivalence would predict the roughly three orders of magnitude ratio of their potencies (331). α- and β-endorphin, the latter about twice the mass of the former per mol, were about equally potent when compared on the basis of weight (R. Guillemin, personal communication, 1983).

The ubiquity of α- and β-sequence short spans in all peptides and proteins (323), the differences in the stability-altering character of the relationships of the two patterns with aqueous solvent, and the five- to ten-fold increase in degrees of freedom in specific amino-acid exchanges using a simple up-down code of variation in hydrophobicities suggest the possibility that the history of difficulty in constructing a scheme for the custom synthesis of peptides (340) may have been due to a requirement for too much specificity. A macro-code of α and β short span rather than amino-acid sequences is suggested. A recent model with this sort of relaxed structural requirement is explained in terms of an α-helical peptide's asymmetric potential for membrane binding (341). The simplest of all possibilities involves a binary code, a dot versus dash transition probability, each successive residue crossing the mean or not in a mod-2 sequence dynamic of hydrophobicity (303). A perfect β-strand would have a $p(\Delta)$ of 1.0, and an α-helical short span, a $p(\Delta)$ of 0.5. The amino-acid sequences of corticotropin-releasing factor and a polypeptide with very similar

actions and potency, urotensin-I (342), differ in 20 of 41 residues. In the binary code of hydrophobicity there are two adjacent transpositions, at 22 and 39, and only two differences, at 27 and 33 (303, 329).

A reflection of the competition for solvent entropy between a macromolecular system (TPOH) and a polypeptide (leu-enkephalin) examined under control conditions and in the presence of the neuropeptide is seen in Figure 16. An $[R\text{-}L]_i$-induced change in the kinetic scattering pattern is seen in a more gaussian distribution of A values from multiple simultaneous determinations, although the median velocity remains the same:

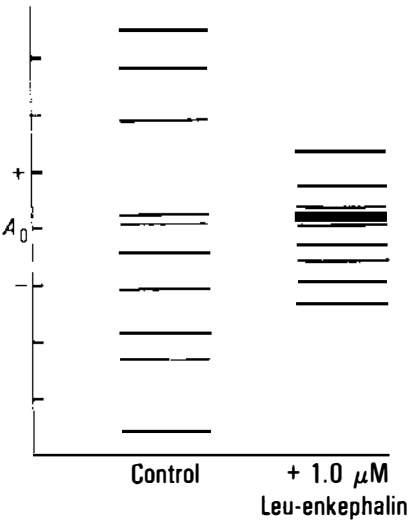

Figure 16

The statistical dynamics of non-equilibrium brain enzyme and receptor systems may offer a new experimental language for studies in molecular psychopharmacology.

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