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The Concept of Allosteric Interaction and Its Consequences for the Chemistry of the Brain

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Throughout this Reflections article, I have tried to follow up on the genesis in the 1960s and subsequent evolution of the concept of allosteric interaction and to examine its consequences within the past decades, essentially in the field of the neuroscience. The main conclusion is that allosteric mechanisms built on similar structural principles operate in bacterial regulatory enzymes, gene repressors (and the related nuclear receptors), rhodopsin, G-protein-coupled receptors, neurotransmitter receptors, ion channels, and so on from prokaryotes up to the human brain yet with important features of their own. Thus, future research on these basic cybernetic sensors is expected to develop in two major directions: at the elementary level, toward the atomic structure and molecular dynamics of the conformational changes involved in signal recognition and transduction, but also at a higher level of organization, the contribution of allosteric mechanisms to the modulation of brain functions.

Theory in Biology

In the physical sciences, there is a long tradition of theory and model building; the advancement of the discipline has relied and still relies on an active interplay between theory and experimental studies and between the development of novel technologies and conceptual invention. In the biological sciences, a similar tradition exists, but it is not as systematic. The frequent enthusiasm for new techniques without explicit theoretical perspectives often hides an impoverished theoretical landscape where original hypotheses or ideas are rare. In my view, the theoretical objectives should precede, or be concomitant with, the technological and experimental means. According to Claude Bernard, "the experimenter who does not know what he is looking for will not understand what he finds" (1).[1] The reasons for the difficulties encountered with the biological sciences possibly lie in the fact that the objects of biology are highly differentiated with multiple, intricate levels of organization. The identification of common conceptual rules further hinges on the considerable structural and functional diversity of the biological objects. Thus, there is the constant dilemma, often split between scientists, about the universality or the singularity of the objects they investigate. In this context, I have selected as a tentative common rule the concept of allosteric interaction and its consequences on the molecular chemistry of proteins, going from bacterial regulatory enzymes up to the nervous system and, tentatively, the higher functions of the brain.

The circumstances of my personal life have been such that as an adolescent, I was already exposed to theory through the teaching of an exceptional professor of natural sciences, Jean Bathellier, who introduced me to the idea of biological evolution. This experience deeply influ-

[1] The full quote (see Ref. 255) is, "Empiricism may serve to accumulate facts, but it will never build science. The experimenter who does not know what he is looking for will not understand what he finds."

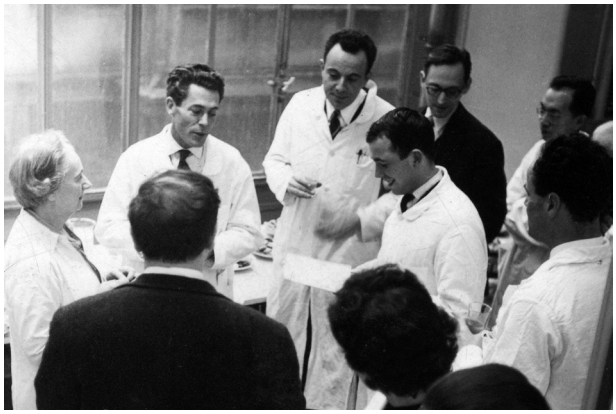

FIGURE 1. **The author reading a poem in 1960 at Jacques Monod's 50th birthday.** From *left* to *right*: Sarah Rapkine, Jacques Monod, François Jacob, the author, and François Gros. The photo was provided by the Fonds d'Archives Madeleine Brunerie at the Institut Pasteur.

enced my scientific career to the extent that until now, as we shall see, my thinking has been framed within the Darwinian evolutionary paradigm. My first laboratory work was in marine biology (2), but I felt rapidly unsatisfied by perspectives essentially limited to descriptive morphology. In the fall of 1958, I visited Jean Brachet's laboratory in Brussels, where I also attended Christian de Duve's lectures and became fascinated by their theoretical perspective that the solution of the great problems of biology had ultimately to be found in elementary biochemical mechanisms. My interest shifted to the more explanatory chemistry of embryonic development. In this context, I imagined a theory in which the enzyme activations that follow the entry of the spermatozoon in the egg were due to a release of enzymes hidden in subcellular particles referred to as lysosomes by Christian de Duve. Back in Paris, I tried to test this hypothesis. Confronted by concrete technical difficulties, I met, by chance, Jacques Monod, who offered to let me enter his laboratory at the Institut Pasteur on the strict condition I abandon my ideas on embryonic development and work with *Escherichia coli*. I drudgingly accepted his demand (3), keeping my theoretical interest in animal biology for the future!

My fascination for theory once again was stimulated in the spring of 1959, when the moment came for the selection of the topic for my Ph.D. thesis. I met Jacques Monod and François Jacob (Fig. 1), who suggested several themes for my research, most of them dealing with their ongoing work on the regulation of gene expression and the operon model. They did not suit me since the theoretical insights and outcomes were already in their hands. An alternative, original topic, mentioned by François Jacob, held my attention. He had heard a talk by Edwin Umbarger, who had shown that in bacterial biosynthetic pathways, such as those for the biosynthesis of L-isoleucine or L-valine, the first enzyme is feedback-inhibited by the end product of the pathway (4). The issue was to understand the molecular mechanism of this "apparently competitive" regulatory interaction, a critical step in the cybernetics of the cell. This topic fitted with my first interest about enzyme activations during fertilization but with a much broader biological perspective. I adopted the theme for my thesis work.

The Concept of Allosteric Interaction (1961–1963)

My first naive idea was that if a special molecular device mediated the feedback inhibition by the enzyme, one should be able to find a way to identify its molecular constituents, for instance, by dissociating the regulatory interaction from the catalytic activity *in vitro*. I first confirmed Umbarger's *in vitro* observations of the apparent competitive inhibition of L-threonine deaminase by L-isoleucine and its "bimolecular" cooperative kinetics toward both the substrate and the feedback inhibitor. I noticed that the sensitivity of enzyme preparations to L-isoleucine changed with the time of storage, purification, heating, and exposure to reagents for –SH groups, resulting in a loss of response to L-isoleucine without a significant decline in enzyme activity. Interestingly, the loss of L-isoleucine feedback inhibition was also accompanied by the abolition of the bimolecular kinetics of the enzyme toward its substrate. The paper I presented at the 26th Cold Spring Harbor Symposium on Quantitative Biology entitled "Cellular Regulatory Mechanisms" (5), at the initiative of Jacques Monod, gave me the stimulating opportunity to theorize. I briefly discussed the two plausible models that might account for the apparently competitive antagonism between the feedback inhibitor L-isoleucine and the substrate L-threonine. According to the first model, the binding sites for the substrate and regulatory inhibitor are partially overlapping, so the interaction is a classical competition by steric hindrance. In the second "new" model, referred to as "no-overlapping," the two sites are separated from each other, and the interaction between ligands takes place between topographically distinct sites. I favored the second model, in which the substrate and regulatory effector were to bind topographically distinct sites, specifically on the basis of the argument that loss of feedback inhibition was accompanied by a normalization of the kinetics (5). Following my presentation, the distinguished bacteriologist Bernard Davis mentioned the possible analogy between the properties of hemoglobin and those of threonine deaminase. As we shall see, it was a highly relevant and inspiring comment (6).

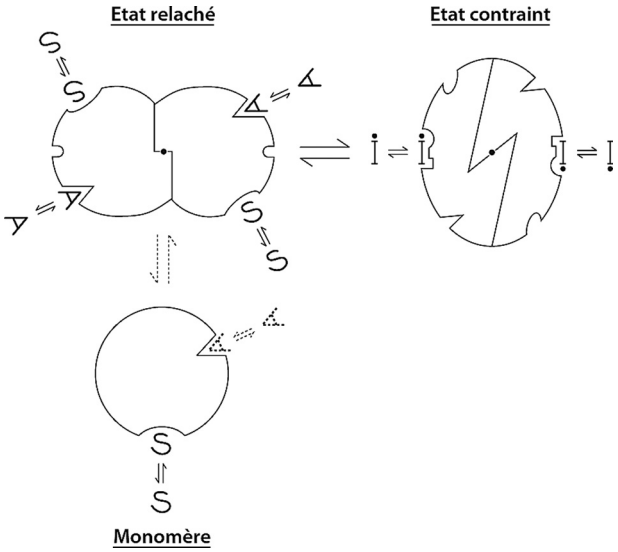

FIGURE 2. **The two-state concerted Monod-Wyman-Changeux model (1965) as originally drawn by the author (Ph.D. thesis, 1964) (73).** *Etat relaché*, relaxed state; *Etat contraint*, constrained state; *Monomère*, monomer.

In the oral presentation of the "General Conclusions" of the symposium, Jacques Monod reported my results and interpretation in the section dealing with the regulation of enzyme activity. He also wrote, "Closely similar observations have been made independently and simultaneously by Pardee (private communication) on another enzyme sensitive to end-product (aspartate-carbamyl-transferase)" (7). In Monod and Jacob's subsequently written "General Conclusions," the word "allosteric" appears for the first time. It is composed of two Greek roots expressing the difference (allo-) in (stereo-) specificity of the two binding sites to qualify and generalize the no-overlapping sites mechanism of indirect interaction between stereospecifically distinct sites mediated by a conformational change of the protein (8).

This was the birth of the word allosteric and of its general definition, which is nowadays widely accepted (8). The introduction of the concept created a major landmark in classical enzymology and the ill-defined notions of "uncompetitive" or "non-competitive" inhibition (9) most often (but not always) assumed to take place in the neighborhood (or even at the level) of the active site.

At this stage, the conformational change linking the topographically distinct sites was interpreted by us in terms of the "induced-fit" theory of Daniel Koshland (10, 11) in the sense that the ligand "instructs" rather than "selects" the structural change (12). At the time, Koshland's concern was not the regulation of enzyme activity by a metabolic signal but the specificity of enzyme action. His theory (11, 13) was that a local steric fit seemed essential for the reaction to occur "only after a change in shape of the enzyme molecule had been induced by the substrate" (13). We suggested the extension of the idea to a higher level long-range and distant allosteric interaction between active and regulatory sites (12). Without being aware of it, we were following the widely accepted tradition of Karl Landsteiner and Linus Pauling's empiricist ideology, viewing the local environment as directly instructing structural changes within biological organisms.

The Monod-Wyman-Changeux Model (1965)

A paradigmatic change from instruction to selection occurred with the Monod-Wyman-Changeux (MWC) model (14). Quite surprisingly, in my opinion, it did not emerge from a deliberate shift of theoretical position by any one of us, but from experimental observations. At the end of 1963, I handed Jacques Monod the first typed version of my thesis work (15, 73, 251–254). Of the many observations I had made, he became especially interested by the experiments I did in 1962 on the effects of urea (16). At an adequate concentration, urea reversibly inactivates L-threonine deaminase, an inactivation interpreted as a split of the enzyme into subunits. Interestingly, in this system, allosteric activators, such as L-norleucine, L-valine, and L-*allo*-threonine, facilitated inactivation and thus subunit dissociation; feedback inhibitors, such as isoleucine, protected against inactivation and thus strengthened the assembly of the subunits in the protein. To formally account for the observed effects, I mentioned the possibility of three possible conformational states, one for each experimental condition (presence of activator, presence of inhibitor, and no effector). Jacques Monod pressed me to systematically adopt in my theoretical reasoning the rule to reduce the number of hypotheses to the strict minimum. Two states should suffice! I immediately agreed and further documented the experimental consequences of the two states with a tight (T) or relaxed (R) mode of packing of the subunits, each state pre-existing the binding of ligand and exhibiting different intrinsic affinities for both substrate and allosteric effectors. In my opinion, this was the birth of the *two-state mechanism* of pre-existing conformational states ($R \leftrightarrow T$), a consequence of a selective, rather than an instructive, effect of the ligands. These views were the start of many debates and theoretical developments that, after many successive writings of the paper by Jacques Monod (17, 18), led to the final version of the MWC model (14).

The model was originally planned to conclude my thesis work with the aim to formally establish a causal link between the structural organization of the known regulatory proteins and their signal transduction properties (Fig.

2). The problem was risky because at that time, the only known three-dimensional structures of proteins were those of hemoglobin (19) and myoglobin (20). Emphasis was placed on the cooperative binding of ligands, a property found frequently associated with the ability to transduce regulatory signals. An essential postulate for us was that cooperativity between ligand-binding sites relies upon the structural cooperativity existing between several subunits in a regulatory protein. In other words, the quaternary assembly of the subunits into a cooperative symmetrical oligomers is critical (although exceptions exist where single folded polypeptides that form several binding sites for different ligands may display allosteric interactions) (21). Max Perutz's structural model of hemoglobin (22, 23) revealed the symmetries of the $\alpha 2\beta 2$-tetramer and the 25–36-Å distance between hemes, together with the evidence that the conformational change that takes place upon O_2 binding primarily concerns the quaternary organization of the molecule. Through several visits, Jeffries Wyman made us aware about his views that the symmetry properties of the O_2 saturation function for hemoglobin reveal the existence of elements of structural symmetry (24). Additional analysis of the assembly of protein oligomers and their symmetry properties was carried out in parallel by Jacques Monod himself and was included in the text.

Along these lines, the MWC model introduces the important notion that signal transduction requires a particular flexibility of protein structure associated with a quaternary constraint established between the subunits within the oligomer, which affects their tertiary organization (and vice versa) (14, 21, 25). Its principal consequence is the all-or-none character of the molecular switch between discrete conformational states, without intermediate hybrid states, associated with cooperative ligand binding. The statement then enunciated put forward that regulatory proteins spontaneously form closed and symmetrical oligomers (or microcrystals) that exist in a few discrete (all-or-none) and symmetrical (R, T…) conformations in thermal equilibrium in the absence of a regulatory signal. The regulatory ligands do not instruct any new adaptative conformations, but merely shift the spontaneous equilibrium between the conformations, selectively stabilizing the one that displays the biological activity and the highest affinity. The model fundamentally distinguishes a function of state "R," which describes the spontaneous conformational equilibrium (defined by its intrinsic equilibrium constant L_0), and a binding function, "Y," which describes the occupation of the binding sites and distinctly evolves as a function of ligand concentration. Several of these statements have since then been reformulated and discussed by several groups in terms of "conformational shift" or "shape shifting" (21, 26–29). They created a theoretical landmark in biochemical thinking by opposing a selectionist model to the traditional induced-fit scheme.

A year following the MWC publication, Koshland, Némethy, and Filmer attempted to rehabilitate the Pauling scheme for O_2 cooperativity in hemoglobin (30) by proposing an instructive model (KNF) (31) that implied a gradual induced change of biophysical parameters with abundant intermediate hybrid states accompanied by the superimposition of the state and binding functions. I took the opportunity to test this prediction during a postdoctoral position with John Gerhart and Howard Schachman. First, I extended the formulation of the MWC model to account for nonexclusive binding of regulatory ligands to both R and T states with the help of Merry Rubin, a computational scientist from the laboratory (32). Then making use of the high amounts of purified aspartate transcarbamylase (7, 33) available for binding experiments (34), together with simultaneously collected conformational data (35), we were able to demonstrate unambiguous differences between the state and binding functions. Moreover, the aspartate transcarbamylase data could be fitted with the general equation of the MWC model (Ref. 36; also see Ref. 37 with phosphofructokinase).

Since the 1960s, abundant structural studies have confirmed the MWC statement that many regulatory devices possess a symmetrical oligomeric structure and undergo the predicted conformational changes (21). The MWC model has also elicited new structural and molecular dynamics investigations in hemoglobin (38–43) and was extended to gene repressors (44, 45), together with the superfamily of nuclear receptors (46–48). We shall see that it was also extended to ion channel-linked (49) and G-protein-linked (50) membrane receptors.

As any scientific theory, the MWC model did not aim to give an exhaustive description of physical reality. It has intrinsic limitations introduced, in particular, by its founding postulates. The formal model was limited to a minimum of two main conformational states, which, as we shall see, are often more numerous (51, 52) and did not include either kinetics and/or molecular dynamics, which was invented after that (29). Also, it did not mention the possible occurrence of regulatory proteins undergoing allosteric transitions as single monomers (21, 29, 52) and the case of some G-protein-coupled receptors (GPCRs) (see "The Nicotinic Receptor: An Authentic Allosteric Membrane Protein"); yet, in my opinion, the major out-

comes of the MWC theory are 2-fold. First, it implements a paradigmatic shift from the cybernetics of biological systems (53, 54) to the molecular mechanism of signal transduction. Second, it has been and still is a strong incentive for empirical research on regulatory proteins using the broad diversity of biophysical methods available.

Cooperativity of Membrane Receptors and the MWC Model

In the conclusion of my Ph.D. thesis in 1964 (15, 73, 251–254), I considered the possibility of extending the MWC model to the "membrane phenomena involved in the recognition of communication signals and their transmission (for example, synaptic transmission)" (73). A few years later, I met Max Delbrück at the California Institute of Technology (Caltech), and he gave me the opportunity to move a step forward in my theoretical reflections and to jump to the level of biological membranes. In September 1966, Max invited me to give a series of three lectures in October at Caltech. Commenting on my last lecture, he was struck by a slide on which I had shown where nearest-neighbor cooperative interactions between allosteric proteins were suggested to take place in a biological membrane. I said that I wanted to develop a mathematical model of such an interaction, and Max suggested that I meet the solid-state physicist Charles Kittel when back in Berkeley. Together with him and a French colleague, Jean Thiéry, the MWC model was re-examined and reformulated. In addition to the classical oligomeric case of membrane receptors, the model was applied to larger, unlimited cooperative assemblies of membrane proteins in a two-dimensional lattice (57). At variance with the MWC model, the thermodynamic formulation was based on the conformational transition of single units (or protomers) between a minimum of two states modulated (or not) by the interaction with other protomers. Depending on the value of the free energy of the interaction between protomers, the model predicts the existence of various classes of responses exhibited by biological systems: from a graded response of a single-receptor protomer or of an oligomeric receptor (MWC model) to an all-or-none phase transition response in large and periodic protein assemblies (56, 57).

The lattice model was rediscovered and successfully developed thirty years later by Dennis Bray and colleagues with the experimental system of bacterial chemoreceptors (58, 59); yet, to date, such cooperative interactions have not been identified in synaptic membranes, only in reconstituted artificial membranes. In contrast, with GPCRs (60–62), the assemblies of homo- and hetero-oligomers of variable sizes as well as between GPCRs and ion channels or ligand-gated ion channels (also referred to as receptor-receptor interactions) have been abundantly documented (63–66).

Other kinds of supramolecular assemblies might possibly be interpreted in terms of adapted versions of the MWC (14) and Changeux *et al.* (57) models such as the chaperonins (67, 68) and possibly the gene transcription complex (69). These models of allosteric interactions have still largely unexplored consequences of the structural and functional organization of supramolecular structures, where the interactions not only take place between ligand-binding sites but also mobilize multiple protein interfaces. In any case, it was an opportunity to reframe the original model in a new biological context.

The Identification of the Nicotinic Acetylcholine Receptor

The MWC model was a fertile inspiration for deeper research into the structural chemistry of proteins, protein design of new functions, and new modulators (21), but I preferred to extend my reflections about membrane cooperativity to carrying the concept of allostery into neuroscience and, to begin with, test the available models on a neural receptor. The John Newport Langley (70) concept of "receptive substance," or receptor theory, was a major landmark in the history of biochemical pharmacology in 1905, yet it was soon criticized and even found "unnecessary" (71) by many experimentalists. Also, for decades, it was believed that neuronal communications in the brain were electrical rather than chemical! No neurotransmitter receptor was biochemically identified until nearly 60 years later (72).

Before leaving the Institut Pasteur in mid-1965, I decided to experimentally challenge the plausible extension of the MWC model to a synaptic protein, the enzyme that degrades the neurotransmitter acetylcholine (ACh), acetylcholinesterase (AChE). I discovered that some of the compounds designed by Daniel Bovet when he was working at the Institut Pasteur (in particular, derivatives of Flaxedil) did not behave as steric competitors but as allosteric modulators of AChE activity (55, 74–76), suggesting a possible but superficial analogy with the authentic receptor.

The time had come to test the MWC model with the true physiological receptor for ACh. A prerequisite was to identify it as a protein! It was achieved by starting from the *Electrophorus electricus* electric organ (72, 77) to use the deliberate strategy to follow the *in vivo* physiological and pharmacological response of a single electroplaque to nic-

otinic agents (77) and to keep these features unmodified *in vitro* at all stages of receptor isolation and chemical identification. I learned to dissect and record from the single electroplaque during a visit of a few months to David Nachmansohn's laboratory at Columbia University in 1967. Back at the Institut Pasteur, I developed an important intermediate assay from purified membrane fragments that make closed microsacs from which radioactive Na^+ (or K^+) ion flux responses to nicotine agonists can be measured. The *in vitro* data displayed ligand specificities that closely resembled those recorded with single electroplaques (78, 79). I used the detergent deoxycholate to gently extract, without denaturation, a protein that reversibly bound the radiolabeled nicotinic agonist decamethonium, using the method of equilibrium dialysis formerly used by Walter Gilbert and Benno Müller-Hill (80) to identify the *lac* repressor. Last, the snake venom toxin α-bungarotoxin, discovered by the Taiwanese pharmacologist Chen-Yuan Lee, was discovered to specifically block the *in vivo* neuromuscular transmission in high vertebrates at the postsynaptic level without interacting with AChE (81). α-Bungarotoxin was found to block altogether the electroplaque's *in vivo* electrical response to nicotinic agonists, the microsac's ion flux response *in vitro*, and the binding of radioactive decamethonium to the detergent extract (82). The extract contained a high molecular weight hydrophobic protein that could then be physically separated from AChE (82) and purified to homogeneity (72, 83).

FIGURE 3. **The author working in his laboratory at the Institut Pasteur in 1987.** This photo was provided by Martine Franck of Magnum Photos.

The Nicotinic Receptor: An Authentic Allosteric Membrane Protein

The secure identification of the nicotinic ACh receptor (nAChR) protein opened a new field of empirical biochemical research (72) motivated by the following question: is the newly identified receptor a *bona fide* allosteric protein? Examination by electron microscopy of the nAChR protein purified from *E. electricus* and from purified nAChR-rich membranes from *Torpedo marmorata* revealed ring-like particles (8–9 nm in diameter) with a hydrophilic core made up of several (five to six) subunits associated in a compact bundle (84–94). Cross-linking of *E. electricus* nAChR further suggested a pentameric organization (95) composed of four types of homologous subunits, $2\alpha1$, $\beta1$, $\gamma1$, and $\delta1$ (96–100), all of which with important sequence identities (99, 101, 102). This finding suggested the possibility, consistent with the MWC model, of a pseudo-symmetrical organization of the nAChR oligomer. The complete sequence of the cDNAs from the several homologous nAChR subunits from the electric organs of *Torpedo californica* (103–106) and *T. marmorata* (107, 108), muscle (109), and brain (110–113) revealed a common organization of all subunits (104, 106, 108) that consisted of a large N-terminal hydrophilic domain, four hydrophobic segments (TM1–TM4), and a small hydrophilic domain. Affinity labeling and site-directed mutagenesis demonstrated that the large hydrophilic domain is extracellular and carries the ACh-binding site (114–118) and that the four hydrophobic segments traverse the transmembrane, with the TM2 segment lining the ion channel (Figs. 3 and 4) (119–122).

The nAChR appeared to behave as an authentic allosteric protein but with particularities of its own (49, 123, 124). 1) It possesses a rather odd oligomeric structure of an homo- or heteropentameric organization, with a C5 axis of rotational symmetry (or quasi-symmetry) perpendicular to the plane of the membrane. 2) The topological distinction between the neurotransmitter (ACh) sites (separated by 40 Å between ACh sites) in the extracellular domain and the ion channel (separated by 60 Å from the ACh sites) in the transmembrane domain unambiguously

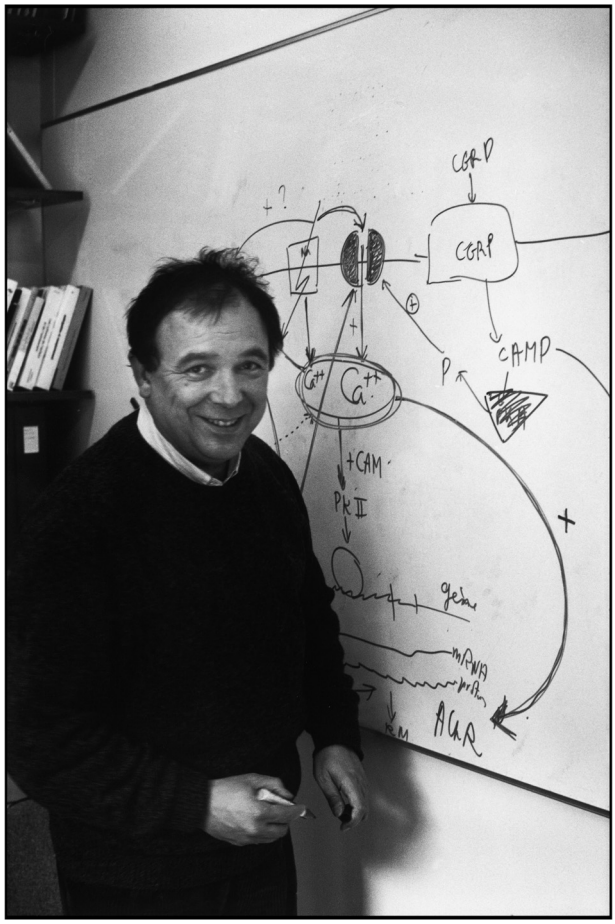

FIGURE 4. **The author (1987) discussing models on the themes of his teaching at the Collège de France (Chair of Cellular Communications).** This photo was provided by Martine Franck of Magnum Photos.

demonstrates that the interaction between the neurotransmitter (ACh) and the channel is an indirect allosteric interaction. 3) Consistent with the MWC model, several discrete conformational changes spontaneously occur in the absence of ligand, but more than two mediate activation (channel opening). The fast and slow inactivations toward higher affinity closed state(s) are referred to as desensitized (49, 125–131). 4) Multiple allosteric "modulatory sites" are present in the synaptic (*e.g.* Ca^{2+}) and transmembrane domain. The anthelmintic ivermectin, general anesthetics, and other molecules regulate these transitions (see below). 5) The conformational equilibrium is shifted by orthosteric and allosteric ligands in favor of the state(s) for which they exhibit a preferential affinity (124–127). Last, constitutive mutations stabilize any one of the accessible conformations (active *versus* inactive) of the nAChR (129, 132–136). Such mutations confer predispositions to human diseases such as congenital myasthenia (129, 133) and autosomal dominant frontal lobe epilepsy (134, 137) (Fig. 5).

The recent discovery that orthologs of nAChRs are present in prokaryotes (138) and behave as ligand-gated ion channels (139) has led to high resolution crystal structures of the receptors from two bacterial species, *Gloeobacter violaceus* (140) and *Erwinia chrysanthemi* (141, 142). They confirm a pentameric symmetrical structure that is strikingly conserved from prokaryotes to eukaryotes (49, 143), thereby opening the exploration of the allosteric transition at the atomic level. Comparison of the x-ray core structures of *Erwinia* and *Gloeobacter* receptors, which are present in closed *versus* open conformations (140–142), revealed a conformational change associated with channel gating manifested by a global quaternary twist around the 5-fold axis of rotational symmetry yet with additional tertiary deformations. An *in silico* normal mode analysis, initially applied to a model of α7-nAChR (144–146), accounts for at least 29% of the allosteric transition deduced from *Erwinia versus Gloeobacter* receptor structures (140–142). The latest ongoing structural developments disclose a novel locally closed structure of the *Gloeobacter* receptor (51), pointing to additional intermediate conformations possibly relevant to the multistep process of desensitization (147).

The isolation of the nicotinic receptor and the demonstration of its allosteric properties were followed by the identification of many members of the pentameric receptor family in the brain (including nAChRs; 5-hydroxytryptamine type 3, $GABA_A$, $GABA_C$, and glycine receptors; and some glutamate, histamine, and 5-hydroxytryptamine-activated anionic receptors) (49, 148–150), as well as other classes of receptors, including the GPCRs (151–153) and channelrhodopsin light-gated cation channels (154).

These studies also pioneered the elucidation at the atomic level of the signal transduction mechanism these receptors mediate and their molecular dynamics (155). In a general manner, the available experimental data favor, but with possible important exceptions, the MWC scheme of pre-existing conformational equilibria (21, 156, 157). However, in several instances such as bias agonism in GPCRs (158), equilibria between multiple conformations (rather than two) have to be taken into consideration (52).

Allosteric Modulation: A New Receptor Pharmacology

Another important consequence of the idea of allostery was the recent re-evaluation of the ancient concept of allosteric modulation (21, 123). It opened new avenues in the pharmacology of the brain and resulted in important applications to drug discovery (158, 159). Early studies

FIGURE 5. **The author in his laboratory at the Institut Pasteur in 1995 with (from *left* to *right*) Alain Bessis, Anne Devillers-Thiéry, Clément Léna, Jean-Luc Eiselé, and Jean-Luc Galzi.** This photo was provided by the Institut Pasteur.

carried out in the 1970s in my laboratory with the aim to identify the binding specificity of various ligands for the nAChR already revealed that channel blockers, like local anesthetics, do not directly displace nicotinic ligands from the ACh-binding site but reversibly bind to a different allosteric site (160–162). The interaction between ACh and local anesthetic sites examined by fluorescence recording was shown to be positive and reciprocal, thus introducing the concept of an allosteric modulatory ligand. Since then, in addition to the main allosteric interaction between neurotransmitter (orthosteric) sites and the ion channel (which binds local anesthetics), multiple allosteric sites to which ligands bind and thus regulate signal transduction have been identified (Fig. 6). One of them was for external Ca^{2+} ions (163, 164), which bind to the extracellular domain of the α7-receptor (165).

Another important type of site for allosteric modulation was discovered in a collaborative work carried out with Daniel Bertrand from the University Medical Center in Geneva (166) using ivermectin. In the micromolar range, this compound strongly enhances the ACh-evoked response of α7-receptors with increased apparent affinity, cooperativity, and maximal response. Based on the alteration of the ivermectin effect caused by mutations in the transmembrane domain (166), it was suggested that ivermectin, like PNU-120596, LY-2087101, and others, acts on α7-nAChRs at the level of a new type of allosteric modulatory site (167, 168), tentatively identified by mutagenesis within the transmembrane domain.

Amazingly, general anesthetics such as propofol and desflurane, which behave as negative modulators of the prokaryotic *Gloeobacter* receptor (169), possess a common binding site identified by x-ray analysis of the *Gloeobacter* receptor within the upper part of the transmembrane domain of each protomer inside a cavity delimited by TM1, TM3, and TM2 within each subunit (169). The cavity of the general anesthetics is accessible from the lipid bilayer, and its entrance is obstructed by a lipid alkyl chain that clashes with propofol binding. Thus, lipids might be the endogenous ligands of this membrane allosteric site (169). These sites appear to be homologous to important modulatory sites of pentameric receptors, such as for ethanol (170, 171), anticonvulsants, anesthetics, and diuretics acting on the glycine or $GABA_A$ receptor or on nAChR, as well present in the transmembrane domain both within and between subunits (172–174).

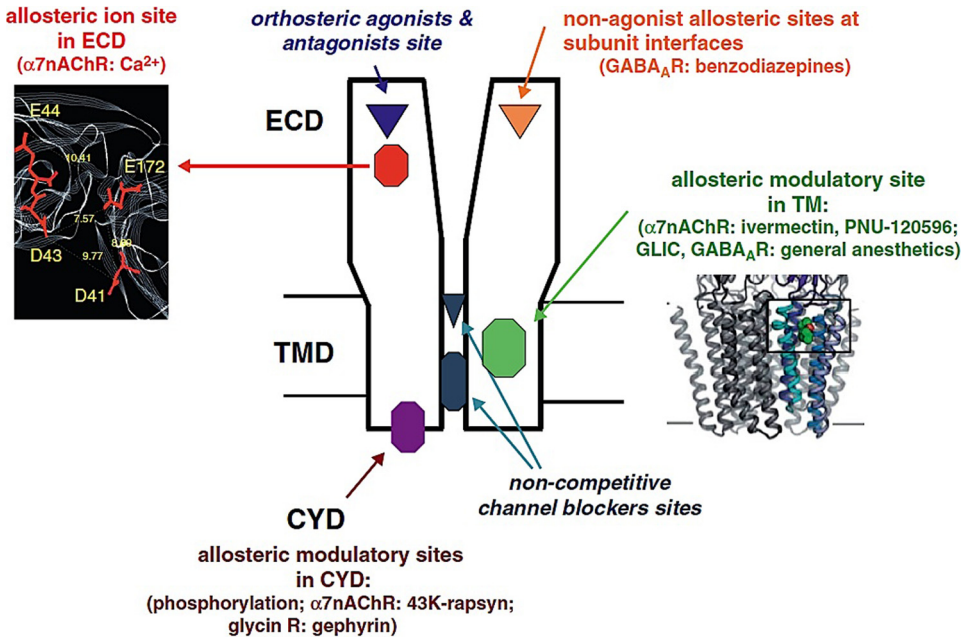

FIGURE 6. **Schematic representation of the various classes of orthosteric and allosteric sites in pentameric ligand-gated ion channels.** Orthosteric sites (shown in *italic blue type*) include the neurotransmitter-binding site and the ion channel. Allosteric sites (shown in various colors) are present in all three domains. *Inset*, *upper left*, model of the allosteric Ca^{2+} site in α-nAChR (from Ref. 146); *lower right*, x-ray structure of the *Gloeobacter* receptor intrasubunit site for general anesthetics (*green*) in the transmembrane domain (Ref. 169; from Ref. 187). *ECD*, extracellular domain; *TMD*, transmembrane domain; *CYD*, cytoplasmic domain; *$GABA_AR$*, $GABA_A$ receptor; *GLIC*, *Gloeobacter* ligand-gated ion channel.

In homopentameric pentamers such as α7-nAChR, there are five active sites per pentamer located at subunit interfaces in the extracellular domain. In heteropentameric neuronal nAChRs such as α4β2-nAChR, not all five homologous sites bind ACh. The non-agonist-binding interface may accommodate, using ACh-binding protein as a model, galantamine, strychnine, cocaine, and morphine at micromolar concentrations (175–177). The possible binding of allosteric modulators at interfaces in nAChRs that do not normally bind nicotinic ligands might be homologous (178) to the important binding site through which benzodiazepines allosterically potentiate $GABA_A$ receptors. Considerable biochemical, pharmacological, and modeling evidence has since then demonstrated that benzodiazepine ligands bind to intersubunit sites in the extracellular domain of the $GABA_A$ receptor that are homologous to the GABA site but do not bind GABA (172–174). Benzodiazepines, after their discovery by Leo Sternbach in 1955, have become the most commonly prescribed psychopharmaceutical drug and the eleventh-most prescribed drug overall, with 46 million prescriptions written for them in 2010 in the United States! Other allosteric modulatory sites are present on the cytoplasmic domain and may play important roles in the clustering, stabilization, and modulation of receptor functions (21, 179, 180, 187).

Without a doubt, the concept of allosteric modulation has created a major landmark in the strategies of drug design for ligand-gated ion channels but also GPCRs, resulting in the successful development of new classes of drugs used in the clinic, such as Gleevec (allosteric inhibitor of Abl tyrosine kinase), cinacalcet (allosteric activator of the calcium-sensing receptor), and maraviroc (allosteric inhibitor of chemokine receptor) (158, 159).

Allosteric Models of Short- and Long-term Synaptic Plasticity

Since my early days in Jacques Monod's laboratory, where I was assigned to work on bacterial proteins, I faithfully remained attached to my adolescent wishes to explore the chemistry of higher organisms. I sought to exploit the knowledge acquired with the chemistry and allosteric properties of the nAChR to progress in the exploration of the well recognized plasticity of brain functions. I realized that to bridge the gap from molecules to cognition, multiple nested levels of organization had to be taken into consideration. Additional theoretical reflections on allosteric receptors were needed, which integrate the molecular, synaptic, neuronal assemblies and cognitive levels.

The first attempt dealt with the elementary process of synapse formation during the postnatal development period, which is especially long in humans (up to 15 years).

In 1970, I had read Jacques Monod's *Chance and Necessity* with great interest but found Monod's position on brain development much too biased by innate influences. As a well informed admirer of Torsten Wiesel and David Hubel's work on the effects of experience on the postnatal development of the visual cortex, I did not share their functional validation hypothesis of preformed innate patterns of nerve connections. The proposal I suggested was that, as an alternative to the classical nativist *versus* empiricist instructive views, an epigenetic "Darwinian" mechanism of synapse selection took place (181–183). It was suggested that overproduced and variable distributions of connections would become transiently established in the course of synapse formation. Then, at a critical or sensitive period, a synapse selection would take place through some kind of trial-and-error process under the control of the electrical and chemical activity of the network (182, 183). As a consequence, some synapses would be stabilized, whereas others, in agreement with earlier observations (184), would regress. The model that was mathematically formalized together with Philippe Courrège and Antoine Danchin (182) had several major consequences. First, it predicted a phenotypic variability of the brain that is found in monozygotic twins. Also, it was an incentive for the search for synapse elimination (frequently called synaptic pruning) and its control by activity. It has since then been recognized in many systems (185, 186)[2] and becomes of critical importance with human babies because of the extended interactions taking place with the social and cultural environment.

The model aroused many and constructive debates (188, 189) but also concrete biochemical investigations, for example, on the differentiation and plasticity of the postsynaptic domain (190, 191), and the discovery that distinct DNA elements (190) and transcription factors (192) control the targeting of receptor transcription under the neuromuscular synapse and its repression by electrical activity outside the end plate, followed by the identification of these various components (124). Finally, post-transcriptional mechanisms, including the assembly of the nAChR by the cytoplasmic 43K-rapsyn protein (87, 193) into supramolecular aggregates, revealed a progressive compartmentalization of the dispersed nAChR at the subjunctional level (194, 195). These studies unveiled the contribution of endogenous trophic factors and electrical activity in the assembly of the subsynaptic membrane (124) and offered a plausible mechanism of long-term synaptic plasticity at the gene expression level.

[2] J.-P. Changeux, manuscript in preparation.

In the chapter "Mental Objects" of *Neuronal Man: The Biology of Mind* (196), inspired by the investigations of the allosteric modulation of the nAChR, I attempted to extend the theory of synapse selection to short-term learning in the brain. In 1982, Thierry Heidmann and I proposed a model for coincident reading of synaptic signals by an allosteric receptor and thus of short-term synaptic efficacy to be able to eventually link individual neurons into cooperative Hebbian assemblies (197). It is based upon the ability of postsynaptic receptors to integrate extra- and intracellular signals through their transmembrane organization via the allosteric modulatory sites and to shift the equilibrium between desensitized and activatable states. This would then determine the amplitude of the change of synaptic efficacy. This mechanism differs from that suggested for the glutamate NMDA receptor, which is based on the voltage-sensitive blocking of a "rigid" ion channel by Mg^{2+} ions; yet it strikingly resembles the proposal by Eric Kandel to account for data from *Aplysia* learning, although the allosteric mechanisms involved were not examined (198, 199).

This allosteric learning mechanism served as an elementary building block (200–202) in the subsequent modeling, realized together with Stanislas Dehaene, of cognitive functions such as the well known delayed-response tasks, which, in mammals, mobilize the prefrontal cortex (203). We proposed that the prefrontal cortex produces variable "anticipations," or hypotheses, as transient patterns of spontaneous neuronal firings (200–202, 204–206). These firings could be selected (*versus* destabilized) at the level of allosteric receptors through the release of a positive (or negative) reward signal evoked by a successful (or unsuccessful) interaction with the outside world (generator of diversity). The neuronal systems specializing in reward and punishment are known to engage defined neurotransmitters and co-existing peptides, including dopamine and serotonin, or ACh/nicotine, together with their receptors, including the nAChR. More elaborate reward-learning algorithms based on predictive Hebbian learning were subsequently reported by other groups (207) and implemented both *in silico* and *in vivo* with monkeys (208, 209). An important consequence of these models, which rely on allosteric receptors, was to establish plausible biochemical links with concrete receptor pharmacology.

Nicotine Addiction, General Anesthesia, and Cognitive Functions

Smoking is the most important preventable cause of mortality and morbidity worldwide. Nicotine, the principal if not sole addictive component of tobacco smoke, exerts reinforcing effects through its action on brain nAChRs

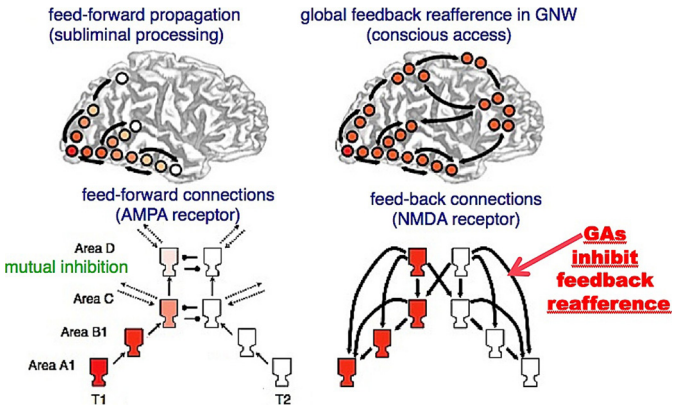

FIGURE 7. **Schematic representation of the hypothesized events leading to conscious access according to the global neuronal workspace model and the action of general anesthetics on cortical reafference (232, 237).** *GNW*, global neuronal workspace; *GAs*, general anesthetics.

together with the laying down of long-term traces in the brain. Their identification and the eventual contribution of allosteric mechanisms to these alterations hinge upon the difficulty that nAChR oligomers are most often composed of several different subunits (sampled among the seventeen ones coded in the human genome) with distinct allosteric properties and are distributed over several brain areas. To identify the patterns of nAChR subunits that contribute to nicotine addiction, new strategies were developed in mice, including nAChR subunit gene deletions, targeted knock-in gene mutations, and re-expression of a deleted gene using stereotaxic injection of a lentiviral vector carrying the missing gene or the relevant siRNA (210–213). The data obtained *in vivo* with these genetically modified mice (214–217) provided evidence that within the dopaminergic neurons from the ventral tegmental area, nAChRs containing combinations of α4-, α3-, α5-, α6-, and β2-subunits mediate the rewarding effects of nicotine administration (215, 218–220). In all of these instances, the different kinds of oligomers involved display characteristic allosteric properties for channel activation, fast and slow desensitization, allosteric modulation, or up-regulation (221–224). These properties thus offer a novel panel of conformational targets for long-term smoking cessation therapies and possibly for alcohol consumption (225).

Addiction is viewed as the end point of a series of transitions from initial voluntary drug use to the "loss of control" over this behavior. Theoretical models (226, 227) and experimental approaches have been suggested for the neuronal bases of drug-elicited loss of control. Smokers with brain damage involving the insula are more likely to quit smoking than smokers with brain damage not involving the insula (228). Also, diffusion tensor imaging studies have revealed that prenatal and adolescent exposure to tobacco smoke alters the development of the microstructure of the white matter, with increased fractional anisotropy in the right and left frontal regions and in the genu of the corpus callosum (229). There is also electrophysiological evidence supporting a direct action of nicotine on axon conduction at the level of nAChRs present at the nodes of Ranvier (230) and at the white matter level (231). These observations support the proposal that nAChRs control a "gating circuit," which itself modulates, in a top-down manner and through the long-range cortical connections of the "global neuronal workspace" (206, 232, 233), nicotine uptake and addiction (227, 234) (Fig. 7). In a general manner, they underline the contribution of ACh and more specifically nAChRs to global "conscious" control (232–235).

In this framework, general anesthesia offers the most vivid evidence for an allosteric modulation of higher brain functions (236, 237). Administration of general anesthetics to patients causes a reversible loss of consciousness manifested, in particular, by a loss of response to oral commands. At appropriate levels of general anesthetics, a deeply unconscious state follows in which the electroencephalogram pattern resembles slow-wave sleep. Parallel biophysical studies on the detailed neural architecture accounting for conscious access called masking experiments (232, 238) suggested the contribution of two distinct sets of connections (239, 240). Bottom-up feedforward short connections link primary sensory areas up to the prefrontal cortex in a cascade. Complementary long-distance top-down connections project from the prefrontal cortex to all preceding areas, creating a top-down global "cortical reafference" (Fig. 7). Quantitative electroencephalogram analysis in humans under propofol anesthesia showed that upon induction, the dominant feedback connectivity of the base-line conscious state was differentially and reversibly inhibited in the frontoparietal network (241). Such selective inhibition would plausibly

FIGURE 8. **The author at the 2009 meeting "Darwin: 200 Years" at Collège de France.** This photo was provided by the Collège de France.

result from a positive allosteric modulation of the cortical $GABA_A$ receptor (among other molecular targets) (242). As mentioned above, these allosteric sites for general anesthetics have been identified in their transmembrane domain (Fig. 6) (169, 172–174). In other words, the loss of consciousness caused by general anesthetic infusion would ultimately result from a modulation of brain allosteric receptors and/or ion channels. The mode of action of general anesthetics on the brain may serve as a concrete illustration of how drugs may allosterically modulate higher brain functions.

Conclusion: The Brain as a Chemical Machine?

In *Neuronal Man: The Biology of Mind* (p. 114 of Ref. 196), I wrote "We must not underestimate the importance of the diversity of the neurons…the multiplicity of the chemical mediators.... (to the extent that) the chemical makeup of the cells that participate in a sensation…is closer to a canvas by Georges Seurat than a composition by Piet Mondrian." This is still very true. The many neurotransmitters and coexisting neuropeptides (243, 244), their multiple receptors (49, 72, 196, 245), which may reach hundreds per cell (246), and their possible combinations are immensely rich and diverse. Also, any individual neuron differs from any other neuron within a given category by its connectivity, its biochemistry, and its physiology due to the developmental variability consecutive to the synapse selection mechanism (182, 196). Confronted with such a complexity, new ideas and modeling enterprises are needed (Fig. 8).

Such a multidisciplinary program is expected to contribute to our understanding of the brain but also to be an incentive for the design and development of technical applications. One of them is optogenetics, which allows the control of the activity of neuronal networks underlying behavior through the millisecond allosteric transition of channelrhodopsin or related photoactivatable molecules (247). Another one of biomedical importance is the design of drugs directed against brain diseases. For instance, it is now recognized that receptors may be present under diverse conformations that spontaneously exist in the absence of ligand. From a drug design perspective, the model suggests that the design of drugs should be targeted to site(s) present on these defined conformation(s) rather than to a single category of rigid binding sites, resulting in the production of agonistic *versus* antagonistic ligands. These studies have also led to the successful discovery of novel allosteric modulators that regulate the activity of ligand-gated ion channels, as well as of other receptors such as GPCRs and tyrosine kinase receptors. Among them are the benzodiazepines, which are the most commonly prescribed psychopharmaceutical drug in the world and which behave as allosteric modulators of $GABA_A$ receptors. The model also predicts that by altering the unliganded equilibrium between discrete conformational states, gene mutations may cause constitutive receptor activation (or inhibition) with important pathological consequences (124, 248). Also, these modeling approaches have enlarged the field of short- and long-term learning to still largely unexplored conformational mechanisms. The "molecular selectionist" schemes of short- and long-term learning, which take place within the genetic envelope of the adult and developing brain, are expected to have important consequences on higher brain functions and their pathologies. Last but not least, the analysis of the patterns of genes expressed in the course of brain development led to the suggestion of a new strategy for designing drugs as orthosteric and allosteric ligands, targeting the transcription factors regulating the "predisposition" genes rather than the proteins they encode (249).

One should not forget in this respect that transcription factors are *bona fide* allosteric proteins.

These theoretical reasonings and data further document and enrich what may be referred to as a "chemical theory of higher brain functions" (196, 250). This creates a striking landmark in the thinking of brain sciences by causally and reciprocally linking the molecular to the cognitive levels both within the individual brain and between brains in the social and cultural environment, thus suggesting new bridges between brain sciences and humanities. From a philosophical point of view, it appears legitimate to say that the future understanding of the mind-brain relationships and the relevant mental processes is likely to rest upon the biochemical world of the allosteric transitions that mediate interneuronal communications through the multiple levels of organization spanning the human brain.

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