Allosteric Receptors: From Electric Organ to Cognition

Jean-Pierre Changeux

Institut Pasteur and Collège de France, Paris, France; email: changeux@noos.fr

Annu. Rev. Pharmacol. Toxicol. 2010. 50:1–38

First published online as a Review in Advance on August 12, 2009

The *Annual Review of Pharmacology and Toxicology* is online at pharmtox.annualreviews.org

This article's doi: 10.1146/annurev.pharmtox.010909.105741

0362-1642/10/0210-0001$20.00

Key Words

nicotinic receptor, allosteric modulation, selective stabilization of synapse, global neuronal workspace

Abstract

This autobiography covers the past 50 years beginning with the development of the concept of allosteric proteins and its application to pharmacological receptors. It continues with the identification of the nicotinic acetylcholine receptor, the discovery of its molecular organization, the structure of the acetylcholine-binding site and of the ion channel, and the demonstration of its allosteric transitions. The article then traces the origins of the concept of allosteric modulator and its consequences in pharmacology. It proceeds with the theory of selective stabilization of synapses and with experimental studies carried out on the contribution of the nicotinic receptor in the morphogenesis of the neuromuscular junction. The knowledge acquired with the nicotinic receptor is further exploited to reach higher levels of brain organization, and the contribution of nicotinic receptors to the action of nicotine on reward and cognition is explored, in particular, using a novel experimental strategy that combines nicotinic receptor genes knock-out and stereotaxic gene re-expression. Theoretical models of cognitive functions are proposed that link the molecular to the cognitive level. The report ends with a discussion on nicotinic receptors and the pharmacology of the future.

INTRODUCTION

The popular view that progress in the accumulation of scientific knowledge occurs by chance events from one spectacular discovery to another may reflect the way media, textbooks, and scientific institutions naively report about the history of science. In reality, within the immense flow of publications, debates, and events that take place in the scientific community, these sharp distinctions look quite artificial. Objective knowledge actually evolves as a network of interactions between an ever-increasing number of scientists distributed throughout the world. It proceeds through the cumulative selection of hypotheses, data, and interpretations, with constant trials and errors, unanticipated encounters, cooperations, imitations, and competitions at the scientific community level. The autobiographical account of a prefatory chapter contrasts with this view in the sense that it is a self-report, invariably subjective and thus necessarily partial. This retrospective synthesis might nevertheless be of some help as a limited individual testimony for a more general historical and comparative reconstruction of the development of concepts and empirical discoveries in pharmacology. Aware of the collective and multidimensional character of this research, I begin this chapter by paying tribute to all collaborators and colleagues, but also insightful competitors, who simply made it possible for this work to take place.

ALLOSTERIC PROTEINS

I was born to a simple and very affectionate French family that was unaware of scientific research. My grandfather was a school teacher in a small village of Rouergue. He communicated to my mother the virtues of education and the outstanding values of secular humanism. I owe to my first mentors, Jean Bathellier, my Natural Sciences Professor at the Lycée Montaigne, and Claude Delamare Debouteville, who opened to me the doors of the Arago's Laboratory of Marine Biology at Banyuls-sur-Mer, for encouraging my adolescent fascination with natural history, and for converting it to a research vocation in life sciences.

In 1954 and 1955, I took summer courses in marine biology at the laboratories of Arcachon and Banyuls-sur-Mer. I had my first contact with the electric discharge of the *Torpedo marmorata* fish and learned about the work of Alfred Fessard and David Nachmansohn on acetylcholine and neurotransmission. However, at this young age, I was more impressed by the diversity of the marine world and was enthusiastic to carry on research on descriptive taxonomy under the tutelage of Claude Delamare Debouteville. My first publications were on the description of a new species and genus of parasitic copepod that I discovered in holothurian echinoderms.

From the taxonomy of parasitic marine copepods to the dawns of molecular biology, the transition was not as abrupt as it may look. I had read with great interest the works of French biologists of the 1930s, such as Lucien Cuénod and Maurice Caullery, in addition to Jean-Baptiste Lamarck and Charles Darwin, and was impressed by their broad theoretical views. My juvenile philosophy, inspired by the work of Jean Brachet and Christian de Duve, whom I had met during a training period of a few weeks in Brussels in the fall of 1958, was that the solution to the great problems of biology were found at the level of elementary biochemical properties and in chemistry. My passion was to understand the evolution of parasites and their unusual embryonic development at the level of the egg cell and the cell's activation by the fertilizing sperm cell that thereby elicits its cleavage and segmentation.

Back in the Institut de Biologie Physico-Chimique in Paris, my plan was to demonstrate that the enzyme activations that follow the entry of the spermatozoon in the egg are due to a burst of lysosomes. Confronted by difficulties in enzyme assays, I asked for help from Jacques Monod, whom I had met by chance in a corridor. Jacques Monod suggested that either I move to the United States or change my topic and enter his lab at the Pasteur Institute. After three months of reflection, I

decided on the latter solution. My arrival in Jacques Monod's laboratory, at the beginning of 1960, put me in the stressful position of testing my rather ambitious ideas with the then-arising methods of molecular biology. Among the several research projects that Jacques Monod and François Jacob proposed for my doctorate thesis, one particularly held my attention. Umbarger and Pardee had independently shown that in certain bacterial biosynthetic pathways the first enzyme is inhibited, in an apparently competitive manner, by the end product of the pathway. The issue was to understand the molecular mechanism of this elementary regulatory operation, which involved two chemical agents, a substrate and a regulatory signal with very different structures. This topic fit directly with the spirit of my first theoretical enthusiasm. I therefore selected it for my thesis subject. The experimentation was difficult for a beginner. I felt rather isolated. I tried hard, with L-threonine deaminase, to find a method to dissociate the regulatory interaction and catalytic activity in vitro. Thiol group reagents, heat treatment, and mutations uncoupled the inhibitory effect caused by isoleucine, while preserving the enzyme's catalytic activity on its substrate. The data contrasted with the classical Michaelis hypothesis of a competitive inhibition. I suggested that the substrate and the regulatory effector were to bind topographically distinct sites (1). In the sketch I drew in the paper I presented at the 1961 prestigious Cold Spring Harbor Symposium on Quantitative Biology, the model of nonoverlapping sites was distinguished from the standard scheme of overlapping sites (i.e., mutual inhibition by steric hindrance) (see **Figure 1**, *top*). The interaction between these two sites was postulated to be indirect and transmitted by a conformational change of the protein molecule (2). The word allosteric was coined by Jacques Monod and François Jacob in the Concluding Remarks of the 1961 Cold Spring Harbor Meeting to qualify and generalize my nonoverlapping sites mechanism. At this stage of the inquiry, the conformational change was viewed as analogous to the induced-fit mechanism suggested long before by Daniel Koshland, not in the case of regulatory interactions but for the catalytic action of enzymes. This was the birth of the concepts of allosteric site and of allosteric interaction that have since been broadly applied to regulatory proteins in general but also to the mode of action of pharmacological agents (see References 3–5).

At the end of my first public presentation of these results at the Cold Spring Harbor meeting, Bernard Davis stood up and noted the analogy between the cooperative binding properties of L-threonine deaminase and oxygen binding to hemoglobin. This was the beginning of an exciting story. My research on the properties of L-threonine deaminase progressed. In particular, I made the critical observation (1), as Gerhart and Pardee (6) independently did, that the sigmoid, cooperative curve of saturation by the substrate was uncoupled by the chemical treatment that dissociated regulatory and active sites. Later (early 1964), I handed Jacques Monod the first version of my thesis work (7). Max Perutz's results on hemoglobin's tridimensional structure as well as Jeffries Wyman's enlightened comments gave rise to vivid reflection and daily debates with my thesis advisor, Jacques Monod. The model that emerged from it, the Monod, Wyman, and Changeux 1965 or MWC model (8), differed from our 1963 views about the conformational change by its selectionist rather than induced-fit character. This shift in reasoning was largely the result of our attempts to relate the discrete signal transduction mechanism elicited by the ligand to the cooperative quaternary organization of the protein molecule and to the cooperativity of its conformational transitions.

It postulated that (see **Figure 1**, *bottom*)

1) regulatory proteins are symmetrically organized from a small number of subunits associated into a cooperative structure forming closed microcrystals, or oligomers, and the tertiary organization of the individual subunits is constrained within the oligomer by its quaternary organization;

2) protein oligomers exist under a few discrete conformations (R-T) in thermal equilibrium in the absence of a regulatory signal;

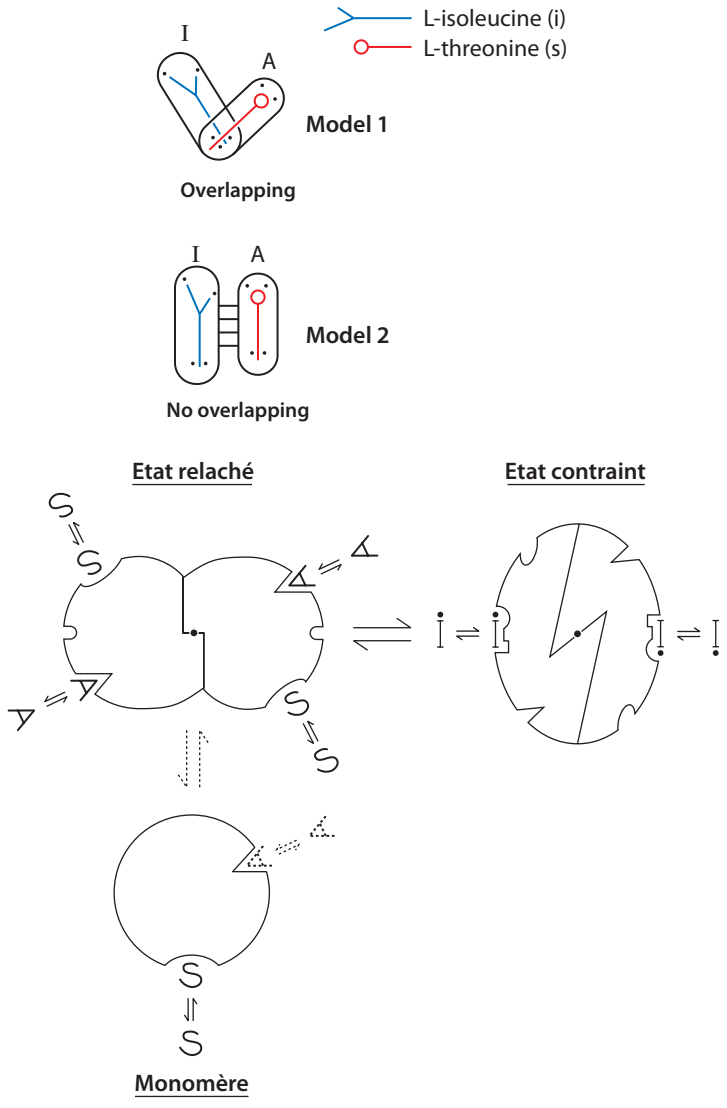

Figure 1

Concepts of allosteric interaction and allosteric transitions. (*Top*) Model of steric hindrance versus no-overlapping sites proposed by Changeux (1) to account for the regulatory interaction mediated between isoleucine and threonine by bacterial L-threonine deaminase. The no-overlapping sites mechanism was named allosteric by Monod & Jacob (1a). (*Bottom*) The two-state concerted model of Monod-Wyman-Changeux (8) as originally drawn by Changeux (7). Translations: etat relaché, relaxed state; etat contraint, constrained state; monomère, monomer.

3) the regulatory ligands merely shift the equilibrium between the conformations, selectively stabilizing the one for which they display the highest affinity; and
4) the conformational transitions take place in an all-or-none concerted fashion for all subunits.

The model accounted jointly for the signal transduction process and for the observed co-operativity between sites without necessarily implying a graded induced change of biophysical parameters.

In 1965, liberated from my military obligations, I decided to further challenge the model and took a postdoctoral position with John Gerhart and Howard Schachman in Berkeley's Molecular Biology Virus Laboratory. They had large amounts of highly purified preparations of aspartate transcarbamylase (ATCase), another bacterial regulatory enzyme. I had in mind to test one of the crucial propositions of the MWC model, that the conformational equilibrium of the protein becomes established independently of the binding of the ligand. Conformation and ligand binding, then, should not vary in parallel as a function of ligand concentration. In other words, the state function *R* should differ from the binding function *Y*. The experiments I did with ATCase demonstrated that this was indeed the case (9). Yet, to fit the data of the original MWC equations, Merry Rubin, a student of Schachman specially competent in computational sciences, helped me to extend the model to the general situation where the ligand nonexclusively binds to both the R and T states (10). This general formulation had two important consequences: (*a*) the new equations adequately fit the ATCase data (despite an uncertainty on the number of sites per ATCase molecule) (11), and (*b*) it offered a plausible mechanism for partial agonism, still an important issue in pharmacology.

Since then, the MWC model has led to considerable structural and biophysical work on the conformational transitions of a large sectrum of regulatory proteins and has become one of the most quoted publications of biochemical literature. Nevertheless, I had to face for decades the challenge of the induced-fit scheme.

The model has been recently re-examined in the framework of molecular dynamics (12) and contrasted again with the sequential induced scheme in particular for hemoglobin (13, 14). The MWC model efficiently accounts for the properties of a broad spectrum of regulatory proteins, including hemoglobin (see References 15, 16), and regulatory enzymes, such as lactate dehydrogenase from *Bifidobacterium* (by X-ray crystallography) (17) or ATCase (by solution nuclear magnetic resonance) (18). The MWC model has become a broadly applied general mechanism of signal transduction by differential stabilization of a (few) discrete state(s) from a pre-existing conformational equilibrium, also referred to as population shifts. Even if more than two conformational states do often occur (19) and if local induced changes adapt the organization of the binding pocket to the actual structure of the ligand, the MWC model has preserved its heuristic value.

In the conclusion of my thesis (1964), I considered explicitly the possibility of extending the MWC model to the "membrane phenomena involved in the recognition of communication signals and their transmission (synaptic transmission, for example)." The theory was further elaborated with the solid-state physicist Charles Kittel during my postdoctoral period at the University of California, Berkeley (1966–1967). In addition to the oligomeric case of membrane receptors, the possibility was considered and expressed in mathematical terms that membrane receptors may form large bi-dimensional arrays of highly cooperative assemblies (20). This extension of the concerted model was documented 30 years later by Dennis Bray (21) with bacterial chemoreceptors. In any case, these reflections on allosteric receptors were the starting point of my investigations on the acetylcholine receptor that I continue today.

Those early years working on allosteric proteins marked my brain synapses for the rest of my life. I became convinced of René Descartes' view that knowledge acquisition relies on theory. Accepted in physics, theory should become, in my opinion, a priority in the biological sciences, which are most often highly empirical and data dominated. Yet, as I shall illustrate, such a top-down theoretical approach needs to be constantly evaluated by bottom-up systematic experimentation with the most advanced technologies. On the other hand, technological progress is necessary but certainly not sufficient for the progress of biological sciences.

IDENTIFICATION OF THE ACETYLCHOLINE RECEPTOR

Before leaving for the United States, I had to satisfy military obligations and was assigned, at the end of 1964, to the laboratory of Claude Paoletti, who made me aware of nerve gas action on acetylcholinesterase, among other topics. A ski accident kept me at home, and I took this opportunity to improve my knowledge of brain sciences and to read, among others, the books of John Eccles (*The Physiology of Synapses* 1964) and David Nachmansohn (*The Chemical and Molecular Basis of Nerve Activity* 1959). Throughout *The Physiology of Synapses*, I became aware of the progress made in the understanding of the physiology of synaptic transmission, in particular the analysis made by Bernard Katz of the ionic phenomena elicited by acetylcholine at the motor endplate. But my interests were closer to the biochemical tradition illustrated by Nachmansohn's attempt to identify the receptor for acetylcholine from the fish electric organ.

At the beginning of the twentieth century, John Newport Langley 1905 (21a) postulated the existence of a receptive substance engaged in the recognition and transduction of drugs or endogenous chemical signals (neurotransmitters) into a physiological response. But the Langley receptor substance had remained a mysterious entity, and the concept itself was heavily criticized by Sir Henry Dale, an established authority in the field. Nachmansohn's book mentioned the early (1959) attempts of Carlos Chagas (22) and Seymour Ehrenpreis (23) from his own laboratory, using as labels either the radioactive curare-like substance flaxedil (gallamine triethiodide) or radioactive curare. Subsequently, Eduardo de Robertis and colleagues (24) used a variety of radioactive cholinergic ligands to characterize a proteolipid extracted from brain membranes and electric tissue by a mixture of chloroform-methanol. These early claims were either withdrawn (25) or seriously challenged and abandoned (See Reference 26 for a review). The debate rebounded with an alternate view that the active site of the enzyme acetylcholinesterase could be the physiological receptor site for the neurotransmitter, a view held by A.O. Zupancic (27), among others. Strong evidence from Nachmansohn's laboratory (28, 29) eliminated this possibility. But could the physiological receptor site be an allosteric site on acetylcholinesterase? I decided to challenge this idea and met, at the Pasteur Institute, France Tazieff-Depierre, who had collaborated with Daniel Bovet when he was working at the Pasteur Institute with Ernest Fourneaux. She gave me several of Bovet's bisquaternary compounds, including flaxedil, to test. Unexpectedly, I found that the structure and properties of acetylcholinesterase from the electric fish *T. marmorata* strikingly vary with ionic strength and that, under the condition of low salt concentrations, the protein carries sites for these bisquaternary compounds, at least partially distinct from the catalytic site (30). These peripheral anionic sites have been extensively studied since (see Reference 31), and the question was raised whether these sites and thus acetylcholinesterase could be part of some kind of allosteric oligomer that would include the receptor transducing unit (30). New methods had to be introduced to answer the question.

On my way to Berkeley, I took a few weeks to continue the work on acetylcholinesterase with Harold Segal in SUNY at Buffalo, to visit Eric Barnard, and to give a seminar for the Nachmansohn laboratory. Once at Berkeley, I remained strongly motivated by the subject. Because the studies on ATCase were successful, I decided to extend my postdoctoral studies with Nachmansohn at Columbia University in New York (1967). During his stay in France at the end of the 1930s, after having fled Nazi Germany, he had discovered the exceptional wealth in biochemical components of the cholinergic synapse of the electric organ from *T. marmorata* and *Electrophorus electricus.* In New York, he had also set up with Ernest Schoffeniels a preparation of individual cells, or electroplaques, isolated from the electric organ, which offered the exceptional opportunity to investigate altogether the electrophysiology, the pharmacology, and the biochemistry of the response to acetylcholine within the same biological system (32). In his laboratory I learned, under the supervision of his

postdoctoral fellow Tom Podleski, to dissect under the microscope, with my own hands, the single electroplaque and to record its electrophysiological response to nicotinic agents. The first electrophysiological data were encouraging: the concentration-response curves had a sigmoid shape and strikingly resembled the cooperative ligand binding curves typical of allosteric enzymes (33).

Jon Singer, whom I had visited a few months earlier at the University of California in San Diego, generously accepted my request for a sample of an affinity-labeling probe that he had previously used with antibodies. This molecule, p-trimethylammonium benzenediazonium fluoroborate (TDF), carries a trimethylammonium group, such as acetylcholine, as weIl as a reactive diazonium group. I was interested in the postulated mechanism that TDF, once bound to the receptor site, would covalently link to the protein. TDF behaved with the electroplaque as expected (34). This was a significant methodological step in the characterization of the receptor; the receptor was amenable to protein chemistry. The method was soon adopted by Arthur Karlin (35), who improved the technique. However, the specificity and the method were still insufficient to allow the isolation of the receptor in its active form from the electric organ.

Back at the Pasteur Institute, where I ran my own laboratory, two discoveries allowed me to overcome this difficulty. First, I benefited from the advice of Maurice Israel, who had been fractionating fish electric organs in the Victor Whittaker laboratory in Cambridge. I was able to separate a purified fraction of membrane fragments rich in acetylcholinesterase from *E. electricus* electric organ that, on electron microscopic sections, formed closed vesicles (36). Inspired by the method used by George Cohen and Jacques Monod that I had practiced with bacterial permeases, I was able, together with my first Japanese postdoctoral fellow Michiki Kasai, to measure radioactive Na^+ (or K^+) ion fluxes with these microsacs by using a simple filtration method. The microsacs responded in vitro with an increase in ion flux in response to nicotinic agonists with a specificity very close to that recorded by electrophysiological methods on the electroplaque. It thus became possible to study in vitro the chemistry of the physiological ionic response to acetylcholine (37).

The receptor molecule was present in the purified membranes, and I immediately tried to label it. I used the agonist, decamethonium, as the radioactive ligand, and I followed its reversible binding by the method of equilibrium dialysis that Gilbert & Müller-Hill used to identify the lac-repressor (38) and that I had experienced in Berkeley with ATCase. The detergent deoxycholate gently extracted the binding protein without denaturing it, and bound decamethonium was displaced by all of the nicotinic agonists and antagonists tested. Yet, it became rapidly evident to me that, even though I was right, if I used only decamethonium and flaxedil as ligands, I would never convince the scientific community that the protein I had isolated was the nicotinic receptor. I nevertheless published the information in the June 8 issue of *Compte Rendus* in 1970 (39). One may note, however, that since then, similar receptor-binding assays have been extensively used by pharmacologists to test an innumerable number of natural or synthetic compounds (40–42).

A second discovery was as decisive. One spring afternoon in 1970, Chen-Yuan Lee, a Taiwanese pharmacologist, unexpectedly came into my laboratory. He informed me of his work on a snake venom toxin, α-bungarotoxin, which he had isolated and purified, and which, according to him, did not interact with acetylcholinesterase but almost irreversibly blocked the neuromuscular junction of higher vertebrates at the postsynaptic level (43). He also mentioned that Dr. Potter (from Miledi's laboratory) was trying to isolate acetylcholine receptor from *T. marmorata*. This did not bother me, because I was convinced that I had already isolated the receptor protein. In any case, aware of Claude Bernard's and Louis Pasteur's lessons in using toxic compounds as chemical lancets to dissect physiological mechanisms, I immediately asked Chen-Yuan Lee for a sample of this toxin, and I tried it as soon as I received it a few days later. The result was remarkable; α-bungarotoxin blocked both the electroplaque's electrical response in vivo and the microsac's

ion-flux response to nicotinic agonists in vitro. It also blocked the binding of decamethonium to the detergent extracts previously solubilized from the microsac preparation. We had the evidence that in the extract was present a protein that binds, in a detergent-soluble form, nicotinic agonists and the snake venom toxin in a mutually exclusive manner. This protein that still reversibly bound the neurotransmitter was shown to have a high molecular weight and to be a hydrophobic protein. It was also mentioned that the protein could be physically separated from acetylcholinesterase. This finding definitively eliminated the possibility that acetylcholinesterase could be part of the receptor complex. The paper appeared in the *Proceedings of the National Academy of Sciences* (communicated by Jacques Monod) (44). Soon after its publication, David Nachmansohn wrote to me, "It is truly magnificient!. . .This time, your experiments seem to me very conclusive that you have successfully isolated the receptor protein. . .Your success opens a new chapter of molecular neurobiology."

The research in my laboratory became more biochemical and molecular. An α-toxin from *Naja nigricollis*, which is closely related to α-bungarotoxin, was provided to us in large amounts by Paul Boquet from the Pasteur Institute, and my first student, Jean-Claude Meunier, was able to covalently couple the toxin to sepharose beads without losing its activity by using the Porath method. Mixing the toxin beads with the membrane extract revealed that 75–100% of the receptor protein bound to the toxin beads, whereas 85–100% of acetylcholinesterase remained in the supernatant. The data, published by Meunier et al. in the January 4 issue of *Compte Rendu* in 1971 (45), confirmed the original intuition of Chen-Yuan Lee but also introduced Cuatrecasas's technique of affinity chromatography to the nicotinic receptor field. Many groups became aware of these new possibilities, among them Edith Heilbronn (46), Edward Reich (47), Jon Lindstrom, and James Patrick (48), who was a postdoctoral fellow in my laboratory when Chen-Yuan Lee visited us. We (49, 50) and others (51) also used alternative affinity columns with immobilized quaternary ammonium agonists or antagonists.

The paper by Miledi et al. (52) finally came out in the February issue of *Nature* in 1971. Unexpectedly, the physiological data were not as convincing as those I obtained with *E. electricus*, possibly because it is difficult to record thin stacks of electroplaques from *T. marmorata*. Miledi et al. used radioactive I^{131}–labeled bungarotoxin (which, according to them, would selectively bind to the receptor in its resting state) to confirm that, in their hands, the separation between acetylcholinesterase and the toxin-binding component occurred following solubilization by 1.5% triton X-100.

Another rather simple-minded technological development that retrospectively had considerable impact on nicotinic receptor research was the isolation of a novel generation of microsacs very rich in receptors. We had in hand a *Naja nigricollis* α-toxin that André Menez had tritiated by his elegant method of catalytic dehalogenation. Aware of the very rich content of the *T. marmorata* electric organ in synaptic material, I asked a new American postdoctoral fellow, Jonathan Cohen, my student Michel Weber, and my technician Monique Huchet (53) to prepare microsacs with *T. marmorata* electric organ instead of *E. electricus*. Surprisingly, on sucrose gradients, the ^{3}H α-toxin labeled a membrane fraction that sedimented faster than the acetylcholinesterase-rich fractions. This fraction appeared to be extremely rich in receptor (20–40% of total protein). The finding was rapidly confirmed by other groups (54, 55). These receptor-rich membranes made the structural and functional properties of the membrane-bound receptor accessible to a variety of biochemical and biophysical methods, such as purification in large quantities (56), fluorescence spectroscopy (57, 58), electron spin resonance (59), and X-ray diffraction (60).

The protein purified from *E. electricus* was examined by Jean Cartaud in parallel with the receptor-rich membranes from *T. marmorata* using the most advanced methods of electron

microscopy then available. The discrete particles (8–9 nm in diameter) had the aspect of a rosette with a hydrophilic core and an asymmetric shape in projection. They resembled a Champagne cork made up of several (5–6) subunits organized into a compact transmembrane bundle (61), and they formed closely packed assemblies in *T. marmorata* postsynaptic membranes (approximately 8–12 000 μm^{-2}) (61). The emotion in the laboratory was intense. For the first time, we could see the structure of a neurotransmitter receptor (61–64).

MOLECULAR ORGANIZATION OF THE ACETYLCHOLINE RECEPTOR AND THE LOCATION OF THE ACETYLCHOLINE-BINDING SITE

It was now possible to display the intimate organization of the receptor molecule. Was it an authentic allosteric protein as I had suggested in the conclusion of my thesis? In other words, was it an oligomeric membrane protein in which a ligand-binding site was able to regulate, via a conformational change, the state of a topologically distinct ionophore site (or channel) through which a flow of ions occurred across the membrane (20, 30, 65)?

First, we had to determine if it was an oligomer, as suggested by the MWC theory. An initial study (66) performed by my first German postdoctoral student, Ferdinand Hucho, by partial cross-linking of the purified *Electrophorus electricus* receptor, revealed a pentameric organization. I was surprised; the theoretical discussions I had had with Jacques Monod in the heroic time of the allosteric enzymes stressed the importance of dyad symmetry axes that, in the case of globular proteins, provided a simple explanation for the evolution of protein monomers into oligomers. Nevertheless, our findings were correct. The teams of Karlin and Raftery, who had no preconceived idea on this particular issue, confirmed the pentameric organization of the receptor but also discovered that the structure was more baroque than we had expected, raising an unanticipated difficulty for the theory. The receptor molecule resulted from the assembly of four apparently quite different types of subunits organized into a $2\alpha.1\beta1\gamma.1\delta$ pentameric oligomer (see Reference 67). These subunits had been distinguished by their molecular mass. Nothing was known about their chemistry.

Again, a new technology was needed. I became aware of the development of a high-resolution microsequencing technique used by my friend, Dony Strosberg. The 20-amino-acid sequence of the α-subunit N-terminal domain was thus established with him (68). Today, this result may look rather modest; however, at the time, it had a significant impact. A chemical identity card of the receptor was henceforth available, the first ever established with a neurotransmitter receptor. It was quickly confirmed by Michael Raftery's team which, with the help of LeRoy Hood's high technology, further determined the N-terminal sequence of the four subunits of the *Torpedo californica* receptor and revealed important sequence identities among the subunits (69). Their finding was a comeback from baroque to classicism. As expected from the MWC theory, the receptor protein was indeed an authentic oligomer, but it was pseudosymmetrical, with an unusual fivefold rotation axis perpendicular to the plane of the synaptic membrane.

The time was ripe to open the nicotinic receptor field to the fast-arising DNA-recombinant technologies. Basing their work on the initial sequence data from us and from Raftery's lab, the teams of Shosaku Numa (70), Stephen Heinemann (71), and Eric Barnard, as well as Anne Devillers-Thiéry and Jérôme Giraudat (72, 73) in my laboratory, struggled to clone the complementary DNAs of the different subunits from electric organ and muscle and to establish their complete sequence. The reading of the sequences revealed several common functional domains along the sequences of the subunits: a long hydrophilic N-terminal segment, four hydrophobic stretches, and a short hydrophilic segment, supposedly organized into extracellular (synaptic), transmembrane, and cytoplasmic domains, respectively. To test the hypothetical allosteric

interaction between distinct sites at the submolecular level, the respective locations of the acetylcholine-binding site and the ion channel had to be determined. Novel information on the actual tridimensional topology of the receptor protein, which was not directly accessible from DNA-recombinant technologies, had to be collected.

In this second step, the method of affinity labeling, which had not enabled the isolation of the receptor, proved to be very useful. A first result was obtained by Karlin's group, which used an affinity-labeling reagent of the acetylcholine-binding site-specific sulfhydryl groups. This led to the identification of a pair of adjacent cysteines (192–193), located in the N-terminal domain of the α-subunit (74). However, this result did not reveal the site's pharmacological specificity.

A new observation brought unexpected insight into the organization of the binding site. It was obtained by photolabeling the (^{3}H) α-toxin-receptor complex by UV irradiation. The γ- and δ-subunits incorporated radioactivity in addition to the α-subunit (75). From this came the idea that the acetylcholine-binding site was located at the interface between subunits (75), which was formerly established for active and regulatory sites with several bacterial regulatory enzymes (15, 17).

The use of DDF, an affinity probe very close to Singer's TDF, which I had used during my stay at Columbia University, brought novel information. The dimethyl ammonium group of DDF created a resonant molecule that could be photoactivated by energy transfer from the protein. Indeed, our team, in collaboration with Christian Hirth and Maurice Goeldner from Strasbourg, identified close to eight amino acids labeled by DDF, six of them with an aromatic side chain and all of them located in the long hydrophilic NH_2 terminal domain of the α-subunit. These amino acids were distributed into three main loops, thus forming a sort of electronegative aromatic basket in which the acetylcholine quaternary ammonium is capable of lodging itself (76, 77). These loops located on the principal component of the binding site were named A, B, and C (78), a nomenclature adopted since then by the receptor community.

In agreement with the photolabeling data with the (^{3}H) α-toxin, DDF labeled the γ- and δ-subunits in addition to the α-subunit (76, 77). The groups of Jonathan Cohen, Palmer Taylor, Arthur Karlin, and ourselves quickly documented this notion and identified loops D, E, and F on the non-α side of the subunit interface. These loops form a complementary component of the acetylcholine-binding site on the γ- and δ-subunits. A first validation of these biochemical results was obtained by directed mutagenesis of the labeled amino acids (77, 78). However, the most spectacular evidence was provided by the Dutch group of Guus Smit & Titia Sixma, using crystallographic analysis of the acetylcholine-binding protein, a soluble snail protein that binds acetylcholine and happens to be homologous to the receptor's extracellular domain (79). Most of the amino acids identified by affinity labeling are precisely found at the acetylcholine-binding-site level and are located at the interface between subunits (compare References 79 and 80).The organizations of the acetylcholine-binding site and of its complex with a variety of nicotinic ligands (for reviews, see References 81–84) thus become accessible for rational drug design at the atomic level (for reviews, see Reference 81), together with the receptor sites from brain nicotinic receptors (85) and from the homologous receptors from the nicotinic family, such as the GABA (86), glycine (87), or 5HT3 receptors (88).

IDENTIFICATION OF THE ION CHANNEL

In the early 1980s, once the complete, one-dimensional sequence of several *Torpedo* species' nicotinic receptor subunits had been established, the most difficult and challenging task remained: the identification of the ion channel! No structure of an ion channel had ever been established. How would it be possible, using the biochemical methods available, to chemically identify a pore within a

protein through which ions flow? The quest (1974–1999) proved to be long and difficult (for review, see Reference 89). Relatively old pharmacological observations, made in Nachmansohn's laboratory and of which I became aware in 1967 during my stay in his laboratory, inspired my search. Pharmacological agents, referred to as local anesthetics, were in fact known to block ion currents activated by nicotinic agonists but in an indirect noncompetitive manner and with no significant competitive effect on the receptor site. These channel blockers acted, as it were, as a cork, and I was convinced they might offer outstanding tools for channel labeling. The first step, performed by Michel Weber and Jonathan Cohen in 1974 with both *E. electricus* and *T. marmorata* receptor-rich membranes, was to demonstrate in vitro that the local anesthetics do not directly displace nicotinic ligands from the acetylcholine-binding site but reversibly bind to a different allosteric site (90, 91). The first attempt of reversible binding with a local anesthetic, quinacrine, pointed toward a protein with a molecular mass of 43,000 Da present in the receptor-rich membrane (92), but it failed. I then decided to tackle the problem using affinity labeling with a covalent local anesthetic synthesized in Bernard Roques' laboratory (93). When we explored the covalent labeling by this photoaffinity probe, Robert Oswald and I (94) noted that UV irradiation of the control molecule, without the reactive group, was sufficient to covalently link the molecule to the receptor δ-subunit. This unanticipated observation enabled us to quickly explore the properties of a large number of potential channel blockers. One of them, chlorpromazine, despite the bad reputation that it lacked specificity, displayed extraordinary properties that were soon recognized by Robert Oswald, me, and a new graduate student, Thierry Heidmann. In *T. marmorata* receptor-rich membranes, chlorpromazine labeled the four types of subunits of the receptor, and this covalent binding was strongly increased by equilibration with nicotinic agonists for all subunits at the same time (94). In addition, the effect of acetylcholine was blocked by d-tubocurarine and α-bungarotoxin. Moreover, accurate measurements made by Thierry Heidmann demonstrated that chlorpromazine binds to just one high-affinity site per $2\alpha.\beta.\gamma.\delta$ oligomer (95). The kinetics of access to this site increased 100-fold when chlorpromazine was rapidly mixed with acetylcholine under conditions expected to open the ion channel (96–98). We thus proposed that chlorpromazine binds to a site located within the ion channel, along the pseudosymmetry axis, that becomes accessible to chlorpromazine when the ion channel opens. The conditions under which the channel could be specifically labeled were thus established. The hardest task remained: to identify the amino acid(s) labeled by chlorpromazine.

It took my student, Jérôme Giraudat, more than a year of relentless efforts to demonstrate that, in the δ-subunit, chlorpromazine specifically labels one amino acid, serine 262, located within the second transmembrane segment (TM2) (99). We were in a state of great excitation. No one had, until then, suggested that the TM2 segment could eventually belong to the ion channel. The results were nevertheless highly reproducible and made public by Jérôme Giraudat at the fall meeting of the Association for Neuroscience in 1985 and were immediately published (99). We were reassured when, months later, Ferdinand Hucho found the same result using the same protocol, but with a different probe (99a). Jérôme Giraudat (100) and later Frédéric Révah (101) shrewdly continued the identification of the chlorpromazine-labeled amino acids on the other subunits. They confirmed, in agreement with Hucho's data, that the serines form a ring, but in addition, they discovered the specific labeling of other amino acids: leucines and threonines located at a distance of three to four amino acids on both sides of the ring of serines. We interpreted that (*a*) the TM2 segments contribute to the channel walls, (*b*) these segments are folded into an α-helix, (*c*) the chlorpromazine-binding site is located at a near-equatorial position in the channel's pseudosymmetry axis, and (*d*) there exists a positive, reciprocal, allosteric interaction between the acetylcholine and chlorpromazine-binding sites.

Shosaku Numa's and Bert Sakmann's teams then used site-directed mutagenesis and single-channel electrophysiological recording techniques—reporting ion transport—after reconstitution

in *Xenopus* oocytes (following the method developed by Barnard & Miledi; see Reference 102) to explore the position of the ion channel in the linear sequence of the δ-subunit. They located in the δ-subunit a region responsible for a conductance difference between *Torpedo* and bovine channels that, according to them, suggests that a region comprising the putative transmembrane segment TM2 and the adjacent bend portion between segments TM2 and TM3 is involved in determining the rate of ion transport through the open channel (103). Subsequent analysis (104) identified rings of negatively charged or glutamine residues, which were referred to as external, intermediate, and cytoplasmic, that frame the amino acid clusters labeled by chlorpromazine, thus confirming my initial proposition that they are located within the ion path.The teams of Henry Lester and Norman Davidson reached in parallel a similar conclusion (105).

Subsequent studies, performed in my laboratory by two postdoctoral fellows, Jean-Luc Galzi and Pierre-Jean Corringer, in collaboration with Daniel Bertrand from the University of Geneva, enabled us to progress further in the ionic selectivity of the channel. We identified a group of three amino acids that drive the conversion of the cationic selectivity of the ion channel into an anionic selectivity. One of the particularly critical amino acids is located in a stretch situated at the cytoplasmic end of the TM2 segment (106, 107). It thus became possible to transform an excitatory acetylcholine receptor into an inhibitory one. This finding, as well as the converse from anionic to cationic, had been reproduced with other receptors: $GABA_A$ (108), glycine (109), GluCl (110), and $5HT_3$ (111). Jean-Luc Eiselé, a Swiss researcher working in my laboratory, successfully constructed a functional chimera that joins the nicotinic receptor synaptic domain and the $5HT_3$ serotonin receptor transmembrane domain (112). Such a chimera (used since by many pharma laboratories for drug-binding assays) unambiguously demonstrated the conservation of tertiary organization between receptor subtypes. Therefore, the structural data obtained with the nicotinic receptor can be generalized to the other receptors of the nicotinic family and are consistent with the recent X-ray structure of a bacterial ligand-gated ion channel (113, 114; see below). They also reveal a rather unconventional location for a stereospecific binding site for pharmacological agents—within a parallel bundle of α-helices!

All of the data clearly indicate that the channel domain is topographically distinct from the acetylcholine-binding domain and thus, as anticipated (7, 20, 30), demonstrates the indirect, allosteric character of the interaction between acetylcholine and the ion transport mechanism.

THE MULTIPLE ALLOSTERIC TRANSITIONS OF THE ACETYLCHOLINE RECEPTOR

Let us go back, for a moment, to the fall of 1967, when I returned to the Pasteur Institute. The acetylcholine receptor was not yet identified, but I nevertheless wanted to test the idea that an allosteric mechanism was involved in the regulation of ion permeability by acetylcholine. Aware of the biochemical work already done with bacterial regulatory enzymes and with hemoglobin, I believed that an efficient way to surpass the current electrophysiological recordings was to directly demonstrate the conformational change that actually mediates this regulation.

In the absence of a specific nicotinic receptor tag, I embarked on fluorescence studies both in vivo and in vitro with nonspecific probes such as 1-anilino 8 naphthalene sulfonate (ANS) with either the whole electroplaque (115, 116) or membranes from *E. electricus* (117). The results were disappointing: no specific responses to nicotinic ligands were recorded. The in vivo measurements monitored the membrane potential, and the in vitro measurements monitored the average states of membrane proteins (e.g., their motion) (118). A visit to Manfred Eigen in 1969 in Göttingen, whom I personally knew for the work he had presented at the Pasteur Institute

on allosteric enzymes, had a deep impact on my thinking. Despite the fact that promising T-jump experiments carried on with his collaborators, Tom Jovin and Rudolf Rigler, on *E. electricus* membranes were unsuccessful, I became convinced that (*a*) the fast kinetic methods pioneered by Eigen were specially adapted to investigate the physiological properties of the nicotinic receptor, and (*b*) specific—instead of nonspecific—probes should be used to follow the conformational dynamics.

As soon as the snake ^{3}H α–toxin and the receptor-rich membranes from *T. marmorata* became available, application of fast mixing methods revealed amazing conformational changes. Electrophysiological recordings had shown that when acetylcholine is applied onto a muscle cell in vivo, a fast (micro- to millisecond) opening of the ion channel, or activation, first occurs, followed by the slow closing (0.01 to several seconds) of the channel, or desensitization (see Reference 119). At equilibrium, acetylcholine binds in a cooperative manner to the receptor sites with a dissociation constant (10 nM) 4–5 orders of magnitude lower than the apparent Kd of the permeability response measured in vitro with the same microsacs (90). Mixing experiments, carried out at that stage by hand, showed that it took seconds to reach this state from a low-affinity resting state (120). Similar findings were subsequently reported with muscle cells (121) and *T. californica* membranes (122). The observation was further documented in the laboratory by Hans Grünhagen, one of Manfred Eigen's former students, with a fluorescent noncompetitive blocker, quinacrine (123, 124). We learned much from the introduction of a fluorescent analog of acetylcholine, dansyl-C_6-choline, synthesized by Bernard Roques and the extensive kinetic analysis of its interaction with receptor-rich membranes with adequate rapid mixing equipment performed by Thierry Heidmann for his thesis (125, 126). The correlation of the binding data with the measurement of ion transport through the ion channel (96) resulted in the first experimental in vitro demonstration of the allosteric transitions of the receptor protein between several conformational states:

1. a resting closed-channel state stabilized by snake α-toxin and nicotinic antagonists,
2. an active, fast, open-channel state with low affinity for acetylcholine and nicotinic agonists, and
3. at least one desensitized, slowly accessible, refractory state, with a high affinity for both agonists and antagonists.

In contrast to a widespread opinion among pharmacologists that the highest affinity states correspond to the active functional state of the receptor, the opposite was true. Consistent with the allosteric scheme, a non-negligible fraction (approximately 20%) of the receptor was spontaneously found in the high-affinity, desensitized state. In parallel studies, Meyer Jackson (127) had observed the spontaneous opening of the muscle receptor in the absence of acetylcholine; the finding was challenged at the time by many physiologists still thinking in terms of induced fit mechanisms, but it has since been confirmed and documented multiple times (see Reference 128). In agreement with the allosteric model, these two observations demonstrated that the transition between low- and high-affinity states of the receptor protein occurs in the absence of acetylcholine and that the bound and unbound open conformations do not significantly differ in their intrinsic binding properties (8, 128). Yet the situation appeared more complex than for regulatory enzymes. There was not only one but a cascade of discrete transitions between open and closed conformational states. This lead Stuart Edlestein, whom I had met in Schachman's laboratory in Berkeley, and me to reformulate the kinetic mechanism of the MWC model for nicotinic acetylcholine receptors on the basis of multiple interconvertible allosteric states (89, 129).

THE QUATERNARY TWIST MECHANISM

In the years following, a wealth of structural information became available from high-resolution electron microscopy of the nicotinic receptor from *Torpedo* (64, 130) and X-ray structure of the acetylcholine-binding protein (82). But little information was available on the structural transitions of the nicotinic receptor, except rather low-resolution electron microscopy studies of *Torpedo* receptor (130) under ill-defined functional conditions (i.e., active or desensitized) (see Reference 131). I thought that in silico modeling might bring useful information on the conformational transitions of the receptor protein. My student, Nicolas Le Novère, began by collecting encouraging secondary structural predictions for nicotinic receptor subunits (132). Then, inspired by the X-ray structure of the molluscan acetylcholine-binding protein, he constructed a three-dimensional model of the amino-terminal extracellular domain and of the acetylcholine-binding pocket of three major types of nicotinic receptors, in particular the brain α7 receptor. Semi-automatic docking of agonists and the α-toxin as an antagonist became possible (133). Yet the α-toxin could not reach its binding site; the loop F region was closing its possible entrance. Molecular dynamics simulation further led to the selection of a conformation appropriate for the α-toxin to penetrate into the receptor, revealing, for the first time, a global reorganization of the protein associated with the allosteric transition of the receptor (134).

Important progress was then realized by Antoine Taly as a postdoctoral fellow in my laboratory. He improved, by comparative analysis, the three-dimensional model of the α7 receptor and performed on it a normal mode analysis that, by approximating the surface of the conformational landscape, gave a decomposition of the protein movements into discrete modes. Among the first 10 lowest frequency modes, the first mode produced a structural reorganization that caused a wide opening of the channel pore resulting from a concerted and symmetrical transition—a quaternary twist motion of the protein with opposing rotations of the upper (extracellular) and lower (transmembrane) domains and significant reorganizations within each subunit at the domain interface. This global quaternary twist motion reasonably accounted for the available experimental data on the gating process (135). This analysis was soon confirmed and extended by several groups (136, 137). Yet we were still missing the relevant X-ray structural information on the whole receptor.

Once more, bacteria helped us immensely! In 2004, I became aware, as a referee, of the finding by Tasneem and colleagues (137a) that sequences of nicotinic receptor homologs, without cytoplasmic domains, could be found in bacterial genomes. Having in mind McKinnon's observations that bacterial membrane proteins are easier to crystallize than eukaryotic ones, I asked Pierre-Jean Corringer, then in my laboratory, whether he would be interested in studying this receptor. He and his student, Nicolas Bocquet, quickly expressed the cyanobacteria *Gloeobacter violaceus* receptor (GLIC) as a homo-pentamer in HEK 293 cells and *Xenopus* oocytes, and they demonstrated that it forms transmembrane cationic channels that are opened by extracellular protons with slow kinetics of activation but without desensitization (138). As anticipated, it was rapidly crystallized at acidic pH, and its X-ray structure revealed an open channel conformation (139, 139a). In parallel, Hilf & Dutzler (140) had elucidated the structure of the homologous receptor from another bacteria, *Erwinia chrysanthemi* (ELIC), which displayed a closed channel. Comparison of the two structures strikingly implemented, for at least 29% of the transition and with a few distinctive differences, the Taly et al. (135) model of the concerted and symmetrical twist opening of the ion channel. This was the first hint at the structural changes mediating signal transduction in a membrane receptor by X-ray crystallography. The data are still limited, but the twist mechanism strikingly resembles (yet with its own particularities, e.g., the symmetry properties) the allosteric transitions identified decades ago by X-ray crystallography with globular allosteric proteins (see References 15, 17).

GAIN-OF-FUNCTION MUTATIONS AND RECEPTOR DISEASES

An unanticipated discovery brought to these structural studies the additional perspective of neurological pathologies. Marc Ballivet and Daniel Bertrand at the University of Geneva, continuing the studies carried out in the laboratories of Patrick, Heinemann, and Lindstrom on brain nicotinic receptors in the 1990s, identified a new subunit type, named α7, in the chick that possessed the remarkable ability to associate with itself into a perfectly symmetrical homomeric receptor (141, 142). Discussing the issue with Marc, I immediately recognized that, because of its simplicity, this system was particularly appropriate to investigate the functional role of the amino acids homologous to those chemically identified by affinity labeling in *T. marmorata*. I asked my student, Frédéric Révah, to specifically mutate the chlorpromazine-labeled amino acids in α7 (143) and collaborated with Daniel Bertrand from Geneva on the project. The first electrophysiological recordings performed by Daniel were amazing. The mutation of leucine 247 to threonine in TM2 did not cause the expected loss of channel function, but on the contrary, resulted in a gain of function: a dramatic decrease in the desensitization rate and a near 100-fold increase in apparent affinity—an astonishing finding! How could it be? While discussing these results at a laboratory meeting, a simple interpretation came to my mind that was based on the three-state allosteric model. Let us assume that, for instance, when the high-affinity desensitized state becomes permeable to ions, desensitization would be abolished. If this were the case, any molecule stabilizing the desensitized state should potentiate the response. I then recalled the studies performed in my laboratory by Hans Grünhagen and the discovery that antagonists, such as curare, could stabilize the desensitized state (123). Thus, we would predict that α7 receptor antagonists, such as dihydro-β erythroidine, could act like agonists. I immediately called Daniel, who did the experiment the same day. Effectively, dihydro-β erythroidine behaved as an agonist on the L247T receptor (144). The 3-state model thus could account simply for the pleiotropic action of the point mutation in the channel domain.

We were more happily surprised when, several years later and in a totally independent way, Andrew Engel, Steven Sine, and their colleagues at the Mayo Clinic in the United States (see Reference 145) reported that in some (not all) patients suffering from congenital myasthenia paralysis, the disorder observed was caused by dominant mutations of the muscle nicotinic receptor that led to a gain of function; some of these mutations (7) are located in MII, and one is precisely at the homologous position of leucine 247. Stuart Edelstein further demonstrated that the properties of these mutant receptors can be adequately fitted, in first approximation, by the allosteric model (146).

The story extends to brain nicotinic receptors. The Australian neurologist Samuel Bercovic recognized that several members of the same family suffered from a rare form of autosomal dominant nocturnal frontal lobe epilepsy, which causes awakenings, losses of consciousness, and convulsions during sleep. Moreover, as shown by the German molecular biologist Ortrud Steinlein, this form of epilepsy resulted from a mutation of the gene coding for the α4 subunit (and also β2) gene(s) of the brain receptor (147). My surprise and delight were even greater when I read in the paper that the particular amino acid whose mutation resulted in seizures was homologous to serine 261 in the MII segment! Spontaneous mutations revealed the same amino acid as the one we had labeled with chlorpromazine to identify the ion channel (99).

The quaternary twist model then offers an interesting structural interpretation of the effect of these mutations (148). Mapping all mutations onto an α7 receptor molecular model revealed that a significant number of them (89%) are found at the interfaces either between subunits or between rigid blocks within subunits, which are regions of the protein where significant changes of conformation are taking place along the quaternary twist model (148). Each individual mutation would

then differentially stabilize either the active (gain-of-function) or the resting (loss-of-function) conformation.

Constitutive mutations have also been reported in a variety of receptor channels, including glycine receptors, GPCRs, tyrosine kinase receptors, and nuclear receptors that might share analogous allosteric properties. A whole class of receptor diseases may thus be directly caused by the perturbation of the allosteric properties of these receptors (for reviews, see References 89, 149).

THE CONCEPT OF ALLOSTERIC MODULATOR: IMPORTANCE FOR PHARMACOLOGY AND LEARNING THEORY

As discussed in the section on the identification of the ion channel, the initial studies carried out in the 1970s with local anesthetics revealed that these compounds do not directly displace nicotinic ligands from the acetylcholine-binding site but reversibly bind to a different allosteric site (90, 91). The interaction between acetylcholine and anesthetic sites was shown to be positive and reciprocal, demonstrating that in vitro pharmacological ligands binding to the site(s) for noncompetitive blockers modulate positively the affinity of the acetylcholine-binding site, thus introducing the concept of allosteric modulatory ligand (for an early discussion, see Reference 150). Subsequent studies showed that the principal high-affinity site for noncompetitive blockers was located in the axis of symmetry within the ion channel. But these studies revealed other categories of sites for local anesthetics, referred to as low-affinity sites, and tentatively suggested that they were located at the interface between the receptor protein and the membrane lipids (95).

Several categories of sites for modulatory ligands of physiological and pharmacological importance were subsequently identified. The first of these ligands was external Ca^{2+} ions. My postdoctoral fellow Christophe Mulle, together with my student Clément Léna, discovered on a preparation of rat medial habenular neurons that external Ca^{2+} concentrations enhance the amplitude of the permeability response to nicotinic agonists via an increased frequency of single-channel openings (151). Galzi et al. (152) further identified the Ca^{2+} site involved by site-directed mutagenesis at the level of the segment covering amino acids 166 to 172 in the vicinity of the complementary moiety of the acetylcholine-binding site. Grafting this segment to the serotonin 5HT3 receptor sequence conferred Ca^{2+} potentiation to this initially Ca^{2+}-insensitive receptor (152). The Ca^{2+} potentiation site has been modelized at the subunit interface (133).

Another kind of positive allosteric modulator of considerable pharmacological importance was discoverd in a collaborative work carried with Daniel Bertrand (153). The antihelmintic ivermectin was found to strongly enhance, in the micromolar range, the acetylcholine-evoked response of $\alpha 7$ nicotinic receptors with increased apparent affinity and cooperativity of the dose-response curve. A reduction of the ivermectin effect was noticed with the L247T mutant. It was suggested that ivermectin acts as a new type of positive allosteric modulator (153). Similar results have been reported subsequently by other groups with synthetic compounds that are efficient cognitive enhancers, such as PNU-120596 and LY-2087101 on $\alpha 7$ nAChRs, and the binding site for these positive modulators has been tentatively identified by mutagenesis within the transmembrane domain at the level of five amino acids within TM1, TM2, and TM4 (154, 154a). These amino acids point toward an intrasubunit cavity located between the four TMs. This site might be common with the binding site for steroids (155) and might be related to the site for volatile anaesthetics within the LGIC family, including $GABA_A$ and glycine receptors (156).

Phosphorylation of the *E. electricus* receptor was discovered by my first Israeli postdoc, Vivian Teichberg (157). Subsequently, my postdoc student Christophe Mulle, looking for a function of the peptide calcitonin-gene-related peptide (CGRP), which in a collaborative work with Thomas Hökfelt (158) was found to coexist with acetylcholine in spinal cord motor neurons, noticed

that CGRP accelerates desensitization of the muscle acetylcholine receptor. This effect operates via an increase of cAMP and stimulation of protein kinase A, plausibly via phosphorylation of the cytoplasmic domain (159). The cytoplasmic domain may thus play important roles in the regulation of receptor function and become a privileged, though largely unexplored, target for drug design.

Another process relevant to allosteric modulation, though still incompletely understood, is the so-called phenomenon of upregulation, through which chronic nicotine exposure elicits a long-term increase in the number of brain high-affinity nAChRs, a paradoxical process that occurs in the brain of smokers (160, 161). The detailed analysis of the process at the cellular level by my student Jerome Salette, working with Pierre-Jean Corringer, revealed that it results from an enhanced maturation and trafficking of the major brain $\alpha4\beta2$ nicotinic receptor (see below) and is initiated in the endoplasmic reticulum soon after protein translation (162, 163). Surprisingly, the nicotinic pharmacology of the site involved differs from that of the mature nicotinic receptor site (163).

These various mechanisms of allosteric modulation of nicotinic receptors may even have influences on learning theory! Inspired by the discovery of the allosteric modulation of the receptor, I proposed in the early 1980s, with Thierry Heidmann (164), a model for a short-term regulation of receptor response, and thus of synaptic efficacy. The hypothetical mechanism is based upon the ability of postsynaptic receptors to integrate extra- and/or intracellular signals through their transmembrane organization via allosteric modulatory sites (see above). This concept of a Hebbian receptor 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. This more general allosteric mechanism has been exploited in formal neuronal networks of cognitive functions (165–167) (see below).

In general, the concept of allosteric modulatory ligand has received considerable interest in receptor pharmacology and drug design, especially for LGICs (3, 168, 169) and GPCRs (4, 5). Its incidence in actual learning processes remains to be evaluated.

FROM THE MOLECULAR TO THE COGNITIVE LEVEL: THEORETICAL CHALLENGES

Parallel to the molecular biology of regulatory enzymes and, later, of the acetylcholine receptor, I was haunted by my adolescent curiosity regarding the chemistry of embryonic development. Seeking to exploit the knowledge aquired with the nicotinic receptor to reach higher levels of organization and function, I realized that this could not be reasonably achieved without efficient theoretical modeling. The following section deals with efforts that cover almost 40 years of research and that, as we shall see, deeply influenced my subsequent work on the nicotinic receptor.

Epigenesis by Selective Stabilization of Synapses

In 1970, Jacques Monod published *Chance and Necessity*. I read the book with great interest but also with critical distance. Although I shared the philosophy, I found his position on the development of the central nervous system too much based on innate influences. A well-informed admirer of Wiesel & Hubel's work on the effects of experience on the postnatal development of the visual cortex (170), I did not share either of their views on the functional validation by experience of preformed innate patterns of nerve connections. At a meeting organized by Edgar Morin, I suggested instead a synaptogenesis mechanism by which exuberant and variable distributions of connections would become transiently established in the course of synapse formation. Then, at a

critical or sensitive period, a synaptic selection would take place according to an epigenetic mode, through some kind of trial-and-error process, under the control of the network activity (171) (see **Figure 2**). If mentioned on several occasions since Ramon y Cajal at the turn of the twentieth century, this concept had been neither mathematically formalized nor put forward as a general theory of learning. Philippe Courrège, Antoine Danchin, and I tried hard to accomplish this (172, 173), and this attempt yielded two major consequences. First, we demonstrated that a particular spatial and temporal distribution of electrical and chemical activity in a developing neuronal network is liable to be inscribed under the form of a particular and stable topology of connections, within the framework of what I called a genetic envelope. Second, the proposition was presented,

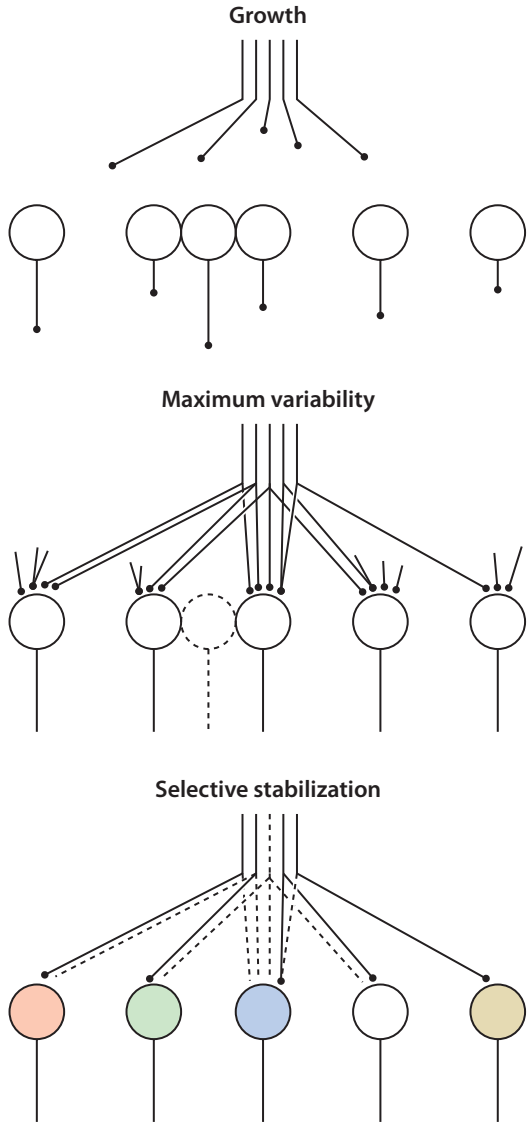

Figure 2

Model of selective stabilization of synapses by Changeux, Courrège, and Danchin (172).

as a theorem of variability, that the selection of networks having different connective topologies can lead to the same input-output behavioral relationship, thus accounting for the constancy of defined behaviors despite brain epigenetic variability.

This theoretical project, according to which an epigenetic evolution by synaptic selection could take over from the genetic evolution of biological species, both at the level of the individual and of the social group, was and still is a major source of inspiration in particular in the genesis and diversification of cultures (see Reference 174). Even if the Darwinian metaphor raises discussions, its application to higher levels of organization, known as the cognitive level, has led to constructive debates with Gerald Edelman, Terrence Sejnowski, Jeff Lichtman, and Dale Purves, among others, and with social scientists in general. As we shall see, these theoretical efforts resulted in abundant experimental studies.

Models of Cognitive Learning by Selection

In 1983, I published *Neuronal Man: The Biology of Mind* that covered the first seven years of lectures I delivered at the Collège de France. In the chapter "Mental Objects," I attempted to extend the theory of epigenesis by synapse selection to higher brain functions. I adopted Hebb's proposal that the representations formed by our brain are activity states of cooperative neuron assemblies. But I integrated it into a selectionist model for knowledge acquisition—instead of the standard instructive mode—thus placing a crucial role on the spontaneous activity of the brain. The neuronal inscription of meaning would be carried out in at least two steps: the spontaneous genesis of multiple and transitory prerepresentations followed by the selection of the adequate representation(s) of the outside world. The first selection mechanism I retained was that of a resonance between prerepresentations of internal origin and the percept evoked by an interaction with external reality (175).

A few years later, a close friend of mine, the psycholinguist Jacques Mehler, put me in contact with one of his students, Stanislas Dehaene, who had received a training in mathematics at the Ecole Normale Supérieure. It was the starting point of a fruitful collaboration that continues today.

Stanislas and I agreed about two major issues from the start: (*a*) we shall elaborate theoretical models with the definite aim to establish causal links between neuronal and behavioral data at an adequate level of organization, and (*b*) the models should be as neuro-realistic as possible and should deal with well-defined behavioral tasks, accessible to experimentation.

The swamp sparrow's song learning, as studied by Peter Marler and his group, was first used as basic material for a network of formal neurons capable of learning sequences of notes through resonance (165). We decided to extend the modeling to more elaborate cognitive functions, such as the well-known delayed-response tasks that, in mammals, mobilize the frontal cortex. The involvement of reward processes, suggested earlier by Edward Thorndike, Ivan Pavlov, or Burrhus Frederic Skinner, looked plausible and adaptable to the selectionist scheme. In addition, the idea enriched the modeling work with a biochemical and pharmacological dimension. Neuronal systems specializing in reward and punishment engaged specific neurotransmitters, such as dopamine and serotonin, as well as acetylcholine or nicotine and thus nicotinic receptors. We developed and formalized the idea (166, 167) that the prerepresentations produced by a neuronal generator of diversity could be selected by the release of a positive reward signal evoked by a successful interaction with the outside world. Conversely, a punishment would destabilize the system and start again the production of prerepresentations. The model included an elementary molecular mechanism of synaptic strength modulation by the reward signal derived from the scheme I proposed with Thierry Heidmann in 1982 for coincidence reading of synaptic signals by

an allosteric receptor (see Reference 164). The virtual organism, constructed on this basis, passed the task. Others were designed for the Wisconsin Card Sorting test (167) or even the Tower of London task (176). The models accounted for the expected cognitive behaviors but also offered many new experimental predictions.

An important feature of these selectionist models was the implementation of evaluation (166) and even auto-evaluation (167, 176) of neurons [see also the algorithms of Barto (177) or Friston (178)] in terms of limbic/mesencephalic aminergic neurons, with which the prefrontal cortex is densely interconnected and to which nicotinic receptors may directly or indirectly contribute. Subsequently, more elaborate reward learning algorithms based on predictive Hebbian learning were reported (179) and implemented both in silico and in vivo in the monkey (180). But the important issue was to establish, through the aminergic neurons, a plausible link between these formal models of cognitive tasks and pharmacology, in particular of the nicotinic receptor (see below).

The Global Neuronal Workspace

Even if anesthesiologists refer without hesitation to loss of consciousness, a fundamental difficulty exists in the use of the word and thus in the research about consciousness in pharmacology and neuroscience. This section should thus be considered the opening of an ongoing discussion aimed at developing empirical research. In *Neuronal Man*, I briefly touched on the issue of consciousness and mentioned in particular the known systems of neuronal regulations that control the global states of the brain in sleep, wakefulness, or dream. In my 1992 course at the Collège de France, I presented the current work of Francis Crick (181), Gerald Edelman (*The Remembered Present*, 1990), Rodolfo Llinas (182), and the relevant controversies within the scientific community. Doing so, I realized that Stanislas Dehaene and myself had been modeling for years tasks requiring a conscious effort. The formal neuronal network that we proposed for the Wisconsin Card Sorting and other cognitive tasks could thus well serve as starting points for the development of a more general model that would include the global workspace suggested by Bernard Baars (183) on psychological grounds and that would account for, altogether, the unitary character of consciousness and the access of a broad diversity of representations to consciousness as well as for the available brain imaging data. The neuronal network suggested (184) (see **Figure 3**) strikingly differed from that of Baars. Instead of referring to the ascending brain stem thalamocortical system, we proposed that neurons with long axons horizontally and reciprocally connecting distinct cortical areas, even different hemispheres, would play an essential role in the genesis of the conscious workspace. Von Economo's studies in the 1920s had further underlined the abundance of pyramidal neurons with long axons in layers II and III of the cerebral cortex. He noted that these layers were especially dense in long-axon neurons in the prefrontal cortex, which also establishes a long range reciprocal circuit with the parietal, temporal, and cingulate areas.

In its initial formulation, the Global Neuronal Workspace Model was applied to the Stroop task, in which a subject is asked to give the color of the ink with which a colored word is printed (184). The task is then extended to the classical perceptual phenomenon referred to as the attentional blink, in which two sensory (i.e., visual) stimuli compete for access to reportable conscious perception (185, 186). The model accounts for a number of brain imaging and electroencephalographic recording studies revealing distinct spatiotemporal patterns of brain activity for conscious versus nonconscious processing (187, 188), thus establishing, in a causal and mechanistic manner, a link between subjective reports and objective physiological recordings.

These theoretical speculations might, at a glance, look far from my empirical work as a pharmacologist; but as I illustrate below, they had a significant impact on the development of my research on the nicotinic receptor.

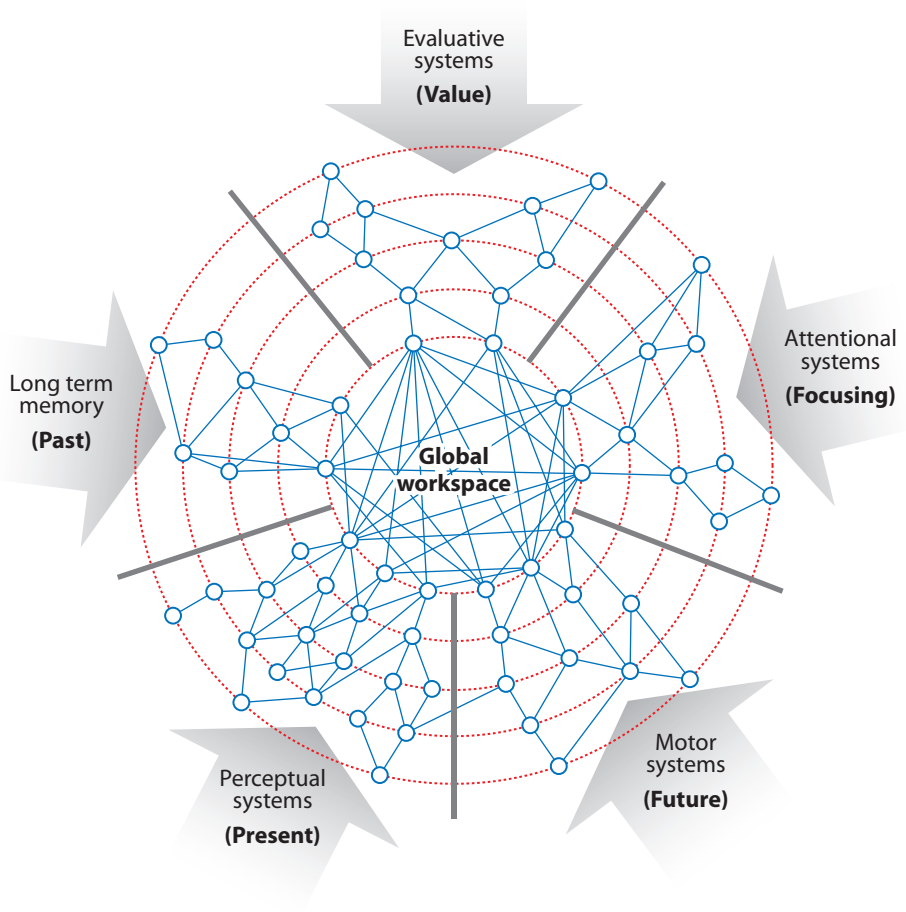

Figure 3

Model of the Global Neuronal Workspace by Dehaene, Kerszberg and Changeux (184).

SUPRAMOLECULAR MORPHOGENESIS OF THE SYNAPSE

The first level of organization to consider beyond the level of the molecule is that of the synapse. I thus decided to directly challenge the model of epigenesis by selective stabilization of synapses. The simplest experimental system available was the neuromuscular synapse, of which the anatomy, physiology, and biochemistry (in particular the acetylcholine esterase and receptor) were known in great detail. At the presynaptic level, Redfern (189) had shown that, during the development of the motor endplate, a multiple innervation with 3–5 nerve endings occurs at birth and disappears later, only one motor axon per muscle fiber persisting in the adult. During his postdoctoral studies in my laboratory in the late 1970s, Pierre Benoit demonstrated, for the first time, that in the newborn rat, the state of activity of the junction controls the elimination of supernumerary terminals (190, 191). Following my suggestion, Francis Crépel and Jean Mariani (192, 194) extended this observation to another interesting model: the cerebellum and the development of the innervation of the Purkinje cells by a single climbing fiber. At birth, a multiple innervation of Purkinje cells by climbing fibers is observed, and this normally regresses during development. In the cerebellum of old X-irradiated

rats and in those of adult weaver, reeler, and staggerer mutant mice, the multiple innervation of Purkinje cells by 3–4 climbing fibers persists up to 18 months (193). In these experimental animals, the pathological motor behavior is more pronounced than in the absence of the cerebellum itself.

Another original aspect of the theory is the suggested role of spontaneous activity on synaptic epigenesis. The anatomical and physiological analysis of the visual system in mice lacking the β2-containing nicotinic receptor (see below), carried out in collaboration with Lamberto Maffei from Pisa, revealed abnormal projection of the retina to the dorsal lateral geniculate nucleus and the visual cortex, which is tentatively assigned to the lack of spontaneous retinal waves (195, 196).

Later, other groups produced, in parallel, experimental data in favor of a selectionist mechanism manifested by what they renamed synaptic pruning (for reviews, see References 197, 198). Recently, these ideas have been reconsidered to explain the pathology of schizophrenia. The current hypothesis is that the pathological phenotype may be due to an enhanced rate of synaptic pruning, resulting in excessive loss of neuronal connectivity in the course of adolescent brain development accompanied with cognitive deficits (199), thus suggesting an entirely new pharmacological approach to schizophrenia based upon synapse stabilization factors.

The model of epigenesis by selection aroused, in parallel, new investigations on the differentiation of the postsynaptic domain on the basis of the knowledge acquired about the molecular biology of the acetylcholine receptor. In particular, α-bungarotoxin allowed early on (in an electron microscopy study performed by my student Jean-Pierre Bourgeois in collaboration with Antoinette Ryter) the evaluation of the number of receptor molecules per unit of postsynaptic membrane surface, showing that their density is extremely high (approximately 15,000 μm^2) and persists, in the electric organ, several weeks after denervation (200, 201). The snake α-toxin became also an exceptional tool in the hands of John Merlie, an American postdoctoral fellow who initiated a fruitful collaboration with François Gros' laboratory (202, 203) and in the hands of Heinrich Betz (my third German postdoctoral fellow), who investigated the expression of the muscle receptor genes and their epigenetic repression by electrical activity during muscle development (204, 205).

A new conceptual stage was reached with the analysis of the genetic determinants that control the regulation of the acetylcholine receptor gene transcription into messenger RNA during the formation of the motor endplate. René Couteaux in the 1960s noticed that the muscle nuclei lying directly under the motor nerve terminal presented a very unusual anatomy, and he named them fundamental nuclei. John Merlie and Josh Sanes (206) also noticed that innervated muscle regions were richer in messenger RNAs coding for the receptor subunits than for the nonjunctional regions. My interest was reinforced by the discovery, made by my medical student Bertrand Fontaine using an in situ hybridization method (developed together with Margaret Buckingham's laboratory) (207, 208), that these messenger RNAs are strictly localized at the level of the fundamental nuclei. There is a compartmentalization of expression of the receptor genes at the level of the subneural domain. Through the years, several students and postdoctoral fellows (André Klarsfeld, 1987; Jacques Piette, 1989, 1990; Jean-Louis Bessereau, 1994; Satoshi Koike, 1996; and Laurent Schaeffer, 1998) actively investigated the genetic mechanisms (DNA elements and transcription factors) that regulate this elementary morphogenesis. We discovered that distinct DNA elements (209–212) and signaling systems differentially control

1. the targeting of receptor transcription under the synapse under the control of a *N Box* (211) element via trophic factors of neural origin and
2. the repression of receptor transcription by electrical activity outside the synapse under the control of an *E Box* element (209, 210).

That led to the identification of an Ets transcription factor, referred to as GABP as critical for the genesis of the postsynaptic membrane (211, 212).

The posttranscription stages (which were studied by Jean Cartaud and colleagues in collaboration with my group) such as the transit through a specialized Golgi apparatus (213), a particular secretory pathway (214), and the assembly by the 43K-Rapsyn protein, a cytoskeletal protein discovered in my laboratory by André Sobel in 1977 (56), into subsynaptic supramolecular aggregates, confirmed and extended the model of a progressive compartmentalization of gene expression in the course of the formation of the neuromuscular synapse.

These studies unraveled genetic processes that mediate the relative contribution of endogenous trophic factors and electrical activity in the supramolecular assembly of a synapse, thus offering a unique mechanism of synaptic plasticity at the molecular level, which might become, one day, the target of an entirely new pharmacology.

BRAIN NICOTINIC RECEPTORS, REWARD, AND NICOTINE ACTION

The regulation of neuronal nicotinic receptor gene expression in the brain involves mechanisms of a much higher level of complexity than the localization of nicotinic receptors at the motor endplate. Clarke, Patrick, Heinemann, and others had described, in the late 1980s, the distinct topological distribution in the brain of the various types of neuronal nicotinic receptors and of the messenger RNAs of their subunits. But I was convinced that to understand the development of such patterns, an efficient strategy was, again, to decipher the network of transcription factors that link dispersed populations of promoter elements in the genome. I first asked Michele Zoli, a postdoctoral fellow from Italy, to carefully examine the expression of the neuronal α3, α4, β2, and β4 genes during development. Their expression starts very early, at day 10 of embryonic development, which is long before the entry of cholinergic innervation and can be synchronous in certain regions (spinal cord) but not in others (cerebral cortex) (215). This differential expression expectedly required a particularly sophisticated transcriptional regulation. My student, Alain Bessis (216, 217), indeed showed that the transcriptional regulation of the highly localized α2 subunit and of the broadly distributed β2 subunit involves complex interplays between activatory and inhibitory regulatory sequences. This was a stimulating start!

A detailed analysis of the posttranscriptional distribution of the nicotinic receptor protein at the level of the nerve cell was performed in parallel using electrophysiological methods by Christophe Mulle (218, 219) and Clément Léna (220). They determined that the receptor protein is distributed not only on the neuronal soma and dendrites but also on the axonal terminals. Another unexpected target of nAChR was found on myelinated axons. The discovery was made through whole-cell recordings of acutely isolated neurons from the interpeduncular nucleus that had retained myelinated fibers still attached to their cell body by synaptic contacts. Nicotine increased the frequency of GABAergic postsynaptic currents on the interpeduncular nucleus neurons, yet unexpectedly, the Na^+ channel blocker tetrodotoxin blocked these effects, revealing that voltage-sensitive Na^+ channels are mobilized in the coupling between nAChR and the GABAergic terminal (220). The term preterminal was coined to qualify the occurrence of these axonal nAChRs, presumably localized at the nœud de Ranvier (see also Reference 221). A possible but still highly speculative function of these axonal receptors would be that they are the target of a global control of acetylcholine on the white matter (222) and thus, possibly, of global states of activity of the brain (i.e., within the global neuronal workspace; see References 184, 185).

Early work on synaptosomal preparations, in particular by the Sue Wonnacott group, identified presynaptic nAChRs in vitro. Yet, their cellular distribution and function in brain networks was not established. In a first electrophysiological approach (223), carried out in the laboratory by Catherine Vidal, field potentials and unit discharges were recorded from the superficial layers of slice preparations of prefrontal cortex. They revealed excitatory effects of nicotine that were

blocked by neuronal bungarotoxin (thus possibly involving α3β2 or most likely α4β2 nAChRs). Furthermore, iontophoretic application of nicotine on pyramidal neurons located predominantly in layers II-III, which belong to the long-axon neurons postulated to be part of the global neuronal workspace (184), revealed a facilitation of glutamatergic excitatory synapses by presynaptic nAChR (224), a finding since confirmed in several laboratories (see Reference 221). Many neurons in the brain have also been found to generate fast inward currents upon application of nicotine to the somatodendritic compartment, in particular cells from the locus ceruleus (225), the ventral tegmental area (226), the medial habenula, and the interpeduncular nucleus (218, 219). Inhibitory GABAergic neurons have also been found to express nAChR, in particular at the postsynaptic level, where they enhance synaptic inhibition (for reviews, see References 226, 227).

Already in the early 1990s, a rich body of pharmacological and behavioral observations underlaid the importance of cholinergic pathways and their nicotinic component in working memory and attentional processes that rely on the contribution of the prefrontal cortex (228, 229). To evaluate the role of nAChRs in cognitive learning in vivo, Sylvie Granon, then in Catherine Stinus–Blanc's laboratory, became associated with us. The effect of nAChR blockers was tested by Granon in the rat on an effortfull delayed-matching-to-sample task (MTS) (230, 231) initially modelized by Stanislas Dehaene and me (166, 167). As anticipated, lesions of the prefrontal cortex selectively impaired the performance of the MTS task (230), and injection of neuronal bungarotoxin into the prelimbic area of the prefrontal cortex produced the same performance deficit. Endogenous acetylcholine acting on nicotinic receptors from the prefrontal cortex was thus necessary for the successful performance of the delayed-response tasks (231).

To further progress in the test of our models and the elucidation of the role of the various types of nicotinic receptors, I decided to shift from the rat to the mouse and to develop an ambitious strategy based on the then-expanding in vivo techniques of gene invalidation (232–236) and selective gene re-expression (237–239).

A first step was the construction by Marina Picciotto, an American postdoctoral fellow, of a mouse invalidated for the β2-subunit, the most largely distributed subunit in the brain (232). The mutant mouse no longer responded to nicotine in a passive avoidance learning task, and also showed alterations in reward processes. Nicotine self-administration as well as the effect of nicotine on dopamine release was abolished (233). The antinociceptive effect of nicotine was also lost, and the same occurred with the α4 mutant mouse (constructed by Lisa Marubio, another American postdoctoral fellow) (234). Loss of the α4- and/or β2-subunit thus dramatically altered nicotine reward/punishment mechanisms.

The spontaneous behavior of the mutant mice was then quantitatively examined by Sylvie Granon. The study revealed in the $\beta2^{-/-}$ mice a shift in favor of navigation to the detriment of a more precise exploration to gather and store spatial information of the environment. That is, the $\beta2^{-/-}$ mice exhibited less behavioral flexibility than the wild-type mice, and they exhibited what might be called a cognitive deficit (236, 237). Moreover, aged $\beta2^{-/-}$ mice show neocortical hypotrophy, loss of hippocampal pyramidal neurons, and impaired spatial learning (236a).

Interestingly, the phenotypes observed with the $\beta2^{-/-}$ mice resemble some of the cognitive impairments observed in human diseases, for instance, in attention deficit hyperactivity disorders (236), in autism (240), or in Alzheimer's disease (236a, 252).

A second step in the strategy was the attempt to map the territories of the brain from a gene-deleted mouse in which the re-expression of the missing gene would restore both the physiological function and the behavior. This could be achieved through the differential expression of a particular nAChR-subunit gene by stereotaxic injection of a vector carrying the gene. By chance, Uwe Maskos, a German postdoctoral fellow already working at the Pasteur Institute, applied to enter my laboratory. He had experience with lentivirus-based expression systems initially developed for

gene therapy purposes (241). These vectors provide stable integration of genes into the genome of nondividing cells, such as neurons. I took this opportunity to ask him to re-express the β2-subunit with a lentiviral vector in the deleted mouse. Stereotaxic injection of a high expression vector into the ventral tegmental area (VTA) resulted in a quantitative re-expression of functional nicotinic receptors in dopamine-containing neurons of the VTA and the full recovery of both nicotine-elicited dopamine release from the nucleus accumbeus (NuAcc) and nicotine self-administration into the VTA (237). Therefore, re-expression of β2-containing receptors in the VTA is not only necessary but also sufficient to re-establish sensitivity to nicotine reward in drug-naive mice. Most interestingly, the lentivirus-injected mice showed a full restoration of exploration, without a significant modification of navigation. These results demonstrate that re-expression of functional β2-containing receptors in the VTA suffices to altogether restore the self-administration of nicotine and the ability to perform an executive behavior under endogenous cholinergic modulation (237), thus bringing experimental evidence in favor of the theoretical mechanisms mentioned above of a causal link between cognition and reward processes (166, 167, 184).

The method thus offers exquisite means to assess the receptor-type specificity of definite neurons in a given behavior. For instance, midbrain dopaminergic ascending pathways are divided into two major tracts: one from the substantia nigra (pars compacta) to the dorsal striatum primarily involved in the regulation of motor activity (its degeneration in humans leads to Parkinson's disease) and another from the VTA to the nucleus accumbens and to the prefrontal cortex pathways mainly implicated as mentioned in cognition and reward. The method was used for a differential rescue of β2-containing receptors in either the substantia nigra (pars compacta) (SNpc) or in the VTA of $\beta2^{-/-}$ mice. Expectedly, SNpc-rescued mice displayed normalized locomotor activity, whereas restoration in the VTA only rescued exploratory cognitive behavior (239). The pharmacological specificity toward nicotinic ligands of the two pathways has been shown to differ (242). The method thus looks efficient enough to dissect the differential role of α4-, α6-, α7-, and β2-subunits in acute systemic self-administration of nicotine (243) but also under conditions of chronic exposure to nicotine (244). The ongoing strategy looks more promising than ever!

NICOTINIC RECEPTORS AND THE PHARMACOLOGY OF THE FUTURE

Another interesting facet of the research on nicotinic receptors is that, unexpectedly, it opened new but still largely unexplored avenues for pharmacology. I mentioned above the theory of the global neuronal workspace for access to consciousness (see Reference 245). Soon, I realized that the theory might suggest plausible interpretations for the fast action of drugs such as nicotine that affect conscious decisions and for their long-term effects leading to addiction (246). The data are still fragmentary, and the interpretation is speculative. Nicotinic ligands, including allosteric modulators (3, 4, 153, 154), may behave as potent cognitive enhancers. Moreover, it is widely accepted that the addictive process consecutive to chronic exposure to nicotine involves a loss of control, an escape from the voluntary control of drug-taking behavior. A plausible hypothesis is that it arises from the disconnection of a reciprocal loop linking the neuronal workspace circuits, including prefrontal cortex, dopaminergic neurons, and striatum, thus uncovering the compulsive nonconscious aspect of drug addiction (for discussion, see Reference 247). Event-related brain-functional MRIs carried out in young adult regular smokers (who have smoked regularly for approximately seven years) and in young adults who never smoke disclosed a reduction of prefrontal attentional network activity in smokers compared with nonsmokers (248). This finding suggests that several years of chronic nicotine abuse may be sufficient to exert long-lasting effects on the neuronal workspace circuits of young adults.

Nicotinic receptors have been shown to control the access to states of vigilance. I had the privilege to establish a collaboration with Hugo Lagercrantz from the Karolinska Institute, who one day visited my lab to find a way to evaluate a possible contribution of nicotinic receptors to sudden infant death syndrome (SIDS). The $\beta 2^{-/-}$ mice became an excellent model of the disease. Recording their state of vigilance by plethysmography (249) first revealed deficits in the arousal from sleep following hypoxic stress (249). Furthermore, newborn mice challenged following exposure to nicotine during pregnancy exhibited impaired arousal. Chronic nicotine exposure thus, similar to the lack of β2-containing nicotinic receptors, impairs the arousal response to distress of newborn mice (250). Consistent with these findings, the prevalence of SIDS is increased severalfold in smoking mothers.

We are still at a very early stage of investigation; yet, these findings, among others (251), illustrate that what may tentatively be referred to as a pharmacology of consciousness has a promising future. In more practical terms, there is considerable interest in the design of pharmacological agents modulating nicotinic receptors to treat nervous system disorders that affect the conscious brain, such as Alzheimer's disease, schizophrenia, depression, attention deficit hyperactivity disorders, and tobacco addiction (252).

CONCLUSION

Throughout this article, which covers almost 50 years of research and is mostly dedicated to the nicotinic receptor, several distinct concepts and empirical discoveries relevant to pharmacology and drug design have been presented and debated, from allosteric proteins, neurotransmitter receptors, and allosteric modulators, to synaptic epigenesis and neuronal workspace. The central hypothesis—that the nicotinic receptor, as a model of membrane receptors, is an allosteric protein—has brought many new insights on the signal transduction mechanism and its consequences for pharmacology. These studies have encompassed several distinct levels of organization within the body and the brain, thus revealing multiple targets for the action and design of pharmacological agents. Under these conditions, many challenges raised by the pharmacologists are far from overcome, but our understanding of the pharmacology of receptors and of the action of medicaments and toxic substances has significantly progressed. A particularly critical issue then becomes to bridge the multiple levels of organization by carefully and efficiently uniting molecular and cellular biology, neuronal networks and the cognitive sciences, opening a promising future for pharmacology and chemical therapeutics. The theoretical approaches become more necessary every day, together with the adequate technologies to evaluate them. But there is still a long way to go.

DISCLOSURE STATEMENT

J.P. Changeux is Emeritus Professor at the Pasteur Institute, a CNRS unit member, a consultant of Servier Laboratory, and a Visiting Professor in the Department of Pharmacology at the University of California, San Diego.

LITERATURE CITED

1. Changeux JP. 1961. The feedback control mechanism of biosynthetic L-threonine deaminase by L-isoleucine. *Cold Spring Harbor Symp. Quant. Biol.* 26:313–18

1a. Monod J, Jacob F. 1961. Teleonomic mechanisms in cellular metabolism, growth, and differentiation. *Cold Spring Harbor Symp. Quant. Biol.* 26:389–401

2. Monod J, Changeux JP, Jacob F. 1963. Allosteric proteins and cellular control systems. *J. Mol. Biol.* 6:306–29
3. Bertrand D, Gopalakrishnan M. 2007. Allosteric modulation of nicotinic acetylcholine receptors. *Biochem. Pharmacol.* 74:1155–63
4. Schwartz TW, Holst B. 2007. Allosteric enhancers, allosteric agonists and ago-allosteric modulators: Where do they bind and how do they act? *Trends Pharmacol. Sci.* 28:366–73
5. Conn PJ, Jones CK, Lindsley CW. 2009. Subtype-selective allosteric modulators of mucarinic receptors for the treatment of CNS disorders. *Trends Pharmacol. Sci.* 30:148–55
6. Gerhart JC, Pardee AB. 1962. The enzymology of control by feedback inhibition. *J. Biol. Chem.* 237:891–96
7. Changeux JP. 1964. On the allosteric properties of L-threonine deaminase. I. Methods of studying biosynthetic L-threonine deaminase. *Bull. Soc. Chim. Biol. (Paris)* 46:927–46; II. Kinetics of action of biosynthetic L-threonine deaminase with respect to the natural substrate and inhibitor. *Bull. Soc. Chim. Biol. (Paris)* 46:947–61; III. Interpretation of the inhibitory effect of L-isoleucine steric hindrance or allosteric effect. *Bull. Soc. Chim. Biol. (Paris)* 46:1151–73; Changeux JP. 1965; IV. The desensitization phenomenon. *Bull. Soc. Chim. Biol. (Paris)* 47:113–39; V. The allosteric transition. *Bull. Soc. Chim. Biol. (Paris)* 47:267–80; VI. General discussion. *Bull. Soc. Chim. Biol. (Paris)* 47:281–300
8. Monod J, Wyman J, Changeux JP. 1965. On the nature of allosteric transitions: a plausible model. *J. Mol. Biol.* 12:88–118
9. Changeux JP, Gerhart JC, Schachman HK. 1968. Allosteric interactions in aspartate transcarbamylase. I. Binding of specific ligands to the native enzyme and its isolated subunits. *Biochemistry* 7:531–38
10. Rubin MM, Changeux JP. 1966. On the nature of allosteric transitions: implications of non-exclusive ligand binding. *J. Mol. Biol.* 21:265–74
11. Changeux JP, Rubin MM. 1968. Allosteric interactions in aspartate transcarbamylase. III. Interpretation of experimental data in terms of the model of Monod, Wyman, and Changeux. *Biochemistry* 7:553–61
12. Cui Q, Karplus M. 2008. Allostery and cooperativity revisited. *Protein Sci.* 17:1295–307
13. Pauling L. 1935. The oxygen equilibrium of hemoglobin and its structural interpretation. *Proc. Natl. Acad. Sci. USA* 21:186–91
14. Koshland DE Jr, Némethy G, Filmer D. 1966. Comparison of experimental binding data and theoretical models in proteins containing subunits. *Biochemistry* 5:365–85
15. Perutz MF. 1989. Mechanisms of cooperativity and allosteric regulation in proteins. *Q. Rev. Biophys.* 22:139–237
16. Eaton WA, Henry ER, Hofrichter J, Bettati S, Viappiani C, Mozzarelli A. 2007. Evolution of allosteric models for hemoglobin. *IUBMB Life.* 59:586–99
17. Iwata S, Kamata K, Yoshida S, Minowa T, Ohta T. 1994. T and R states in the crystals of bacterial L-lactate dehydrogenase reveal the mechanism for allosteric control. *Nat. Struct. Biol.* 1:176–85
18. Velyvis A, Yang YR, Schachman HK, Kay LE. 2007. A solution NMR study showing that active site ligands and nucleotides directly perturb the allosteric equilibrium in aspartate transcarbamoylase. *Proc. Natl. Acad. Sci. USA* 104:8815–20
19. Lange OF, Lakomek NA, Farès C, Schröder GF, Walter KF, et al. 2008. Recognition dynamics up to microseconds revealed from an RDC-derived ubiquitin ensemble in solution. *Science* 320:1471–75
20. Changeux JP, Thiéry JP, Tung Y, Kittel C. 1967. On the cooperativity of biological membranes. *Proc. Natl. Acad. Sci. USA* 57:335–41
21. Bray D, Levin MD, Morton-Firth CJ. 1998. Receptor clustering as a cellular mechanism to control sensitivity. *Nature* 393:85–88

21a. Langley JN. 1905. On the reaction of cells and of nerve-endings to certain poisons, chiefly as regards the reaction of striated muscle to nicotine and to curari. *J. Physiol.* 33:374–413

22. Chagas C. 1959. Studies on the mechanism of curarization. *Ann. N. Y. Acad. Sci.* 81:345–57
23. Ehrenpreis S. 1959. Interaction of curare and related substances with acetylcholine receptor-like protein. *Science* 129:1613–14
24. De Robertis E, Fiszer S, Soto EF. 1967. Cholinergic binding capacity of proteolipids from isolated nerve-ending membranes. *Science* 158:928–29

25. Ehrenpreis S. 1964. Acetylcholine and nerve activity. *Nature* 201:887–93
26. Briley MS, Changeux JP. 1977. Isolation and purification of the nicotinic acetylcholine receptor and its functional reconstitution into a membrane environment. *Int. Rev. Neurobiol.* 20:31–63
27. Zupancic AO. 1953. The mode of action of acetylcholine; a theory extended to a hypothesis on the mode of action of other biologically active substances. *Acta Physiol. Scand.* 29:63–71
28. Podleski TR. 1967. Distinction between the active sites of acetylcholine-receptor and acetylcholinesterase. *Proc. Natl. Acad. Sci. USA* 58:268–73
29. Karlin A, Bartels E. 1966. Effects of blocking sulfhydryl groups and of reducing disulfide bonds on the acetylcholine-activated permeability system of the electroplax. *Biochim. Biophys. Acta* 126:525–35
30. Changeux JP. 1966. Responses of acetylcholinesterase from *Torpedo marmorata* to salts and curarizing drugs. *Mol. Pharmacol.* 2:369–92
31. Silman I, Sussman JL. 2008. Acetylcholinesterase: How is structure related to function? *Chem. Biol. Interact.*175:3–10
32. Schoffeniels E, Nachmansohn D. 1957. An isolated single electroplax preparation. I. New data on the effect of acetylcholine and related compounds. *Biochim. Biophys. Acta* 26:1–15
33. Changeux JP, Podleski TR. 1968. On the excitability and cooperativity of the electroplax membrane. *Proc. Natl. Acad. Sci. USA* 59:944–50
34. Changeux JP, Podleski TR, Wofsy L. 1967. Affinity labeling of the acetylcholine-receptor. *Proc. Natl. Acad. Sci. USA* 58:2063–70
35. Karlin A, Winnik M. 1968. Reduction and specific alkylation of the receptor for acetylcholine. *Proc. Natl. Acad. Sci. USA* 60:668–74
36. Changeux JP, Gautron J, Israel M, Podleski TR. 1969. Separation of excitable membranes from the electric organ of *Electrophorus electricus*. *C. R. Acad. Sci. Hebd. Seances Acad. Sci. D.* 269:1788–91
37. Kasai M, Changeux JP. 1971. In vitro excitation of purified membrane fragments by cholinergic agonists. *J. Memb. Biol.* 6:1–23; 4–57; 58–80; 81–88
38. Gilbert W, Müller-Hill B. 1966. Isolation of the lac repressor. *Proc. Natl. Acad. Sci. USA* 56:1891–98
39. Changeux JP, Kasai M, Huchet M, Meunier JC. 1970. Extraction from electric tissue of gymnotus of a protein presenting several typical properties characteristic of the physiological receptor of acetylcholine. *C. R. Acad. Sci. Hebd. Seances Acad. Sci. D* 270:2864–67
40. Pert CB, Snyder SH. 1973. Opiate receptor: demonstration in nervous tissue. *Science* 179:1011–14
41. Terenius L. 1973. Stereospecific interaction between narcotic analgesics and a synaptic plasma membrane fraction of rat cerebral cortex. *Acta Pharmacol. Toxicol. (Copenh)* 32:317–20
42. Snyder SH. 2004. Opiate receptors and beyond: 30 years of neural signaling research. *Neuropharmacology* 47(Suppl 1):274–85
43. Lee CY, Chang CC. 1966. Modes of actions of purified toxins from elapid venoms on neuromuscular transmission. *Mem. Inst. Butantan.* 33:555–72
44. Changeux JP, Kasai M, Lee CY. 1970. Use of a snake venom toxin to characterize the cholinergic receptor protein. *Proc. Natl. Acad. Sci. USA* 67:1241–47
45. Meunier JC, Huchet M, Boquet P, Changeux JP. 1971. Separation of the receptor protein of acetylcholine and acetylcholinesterase. *C. R. Acad. Sci. Hebd. Seances Acad. Sci. D* 272:117–20
46. Karlsson E, Heilbronn E, Widlund L. 1972. Isolation of the nicotinic acetylcholine receptor by biospecific chromatography on insolubilized *Naja naja* neurotoxin. *FEBS Lett.* 28:107–11
47. Klett RP, Fulpius BW, Cooper D, Smith M, Reich E, Possani LD. 1973. The acetylcholine receptor. I. Purification and characterization of a macromolecule isolated from *Electrophorus electricus*. *J. Biol. Chem.* 248:6841–53
48. Lindstrom J, Patrick J. 1974. Purification of the acetylcholine receptor by affinity chromatography. In *Synaptic Transmission and Neuronal Interaction*, ed. MVL Bennett, pp. 191–216. New York: Raven Press
49. Olsen R, Meunier JC, Changeux JP. 1972. Progress in purification of the cholinergic receptor protein from *Electrophorus electricus* by affinity chromatography. *FEBS Lett.* 28:96–100
50. Meunier JC, Sealock R, Olsen R, Changeux JP. 1974. Purification and properties of the cholinergic receptor protein from *Electrophorus electricus* electroplax. *Eur. J. Biochem.* 45:371–94
51. Schmidt J, Raftery MA. 1972. Use of affinity chromatography for acetylcholine receptor purification. *Biochem. Biophys. Res. Commun.* 49:572–78

52. Miledi R, Molinoff P, Potter LT. 1971. Isolation of the cholinergic receptor protein of *Torpedo* electric tissue. *Nature* 229:554–57
53. Cohen JB, Weber M, Huchet M, Changeux JP. 1972. Purification from *Torpedo marmorata* electric tissue of membrane fragments particularly rich in cholinergic receptor. *FEBS. Lett.* 26:43–47
54. Duguid JR, Raftery MA. 1973. Fractionation and partial characterization of membrane particles from *Torpedo californica* electroplax. *Biochemistry* 12:3593–97
55. Schiebler W, Hucho F. 1978. Membranes rich in acetylcholine receptor: characterization and reconstitution to excitable membranes from exogenous lipids. *Eur. J. Biochem.* 85:55–63
56. Sobel A, Weber M, Changeux JP. 1977. Large-scale purification of the acetylcholine-receptor protein in its membrane-bound and detergent-extracted forms from *Torpedo marmorata* electric organ. *Eur. J. Biochem.* 80:215–24
57. Cohen JB, Changeux JP. 1973. Interaction of a fluorescent ligand with membrane-bound cholinergic receptor from *Torpedo marmorata*. *Biochemistry* 12:4855–64
58. Grünhagen H, Changeux JP. 1975. Structural transitions of the *Torpedo* cholinergic receptor in its membrane state as demonstrated by a fluorescent local anesthetic: quinacrine. *C. R. Acad. Sci. Hebd. Seances Acad. Sci. D* 281:1047–50
59. Brisson AD, Scandella CJ, Bienvenue A, Devaux P, Cohen JB, Changeux JP. 1975. Interaction of a spin-labeled long chain acylcholine with the cholinergic receptor protein in its membrane environment. *Proc. Natl. Acad. Sci. USA* 72:1087–91
60. Dupont Y, Cohen JB, Changeux JP. 1974. X-ray diffraction study of membrane fragments rich in acetylcholine receptor protein prepared from the electric organ of *Torpedo marmorata*. *FEBS Lett.* 40:130–33
61. Cartaud J, Benedetti L, Cohen JB, Meunier JC, Changeux JP. 1973. Presence of a lattice structure in membrane fragments rich in nicotinic receptor protein from the electric organ of *Torpedo marmorata*. *FEBS Lett.* 33:109–13
62. Ross MJ, Klymkowsky MW, Agard DA, Stroud RM. 1977. Structural studies of a membrane-bound acetylcholine receptor from *Torpedo californica*. *J. Mol. Biol.* 116:635–59
63. Brisson A, Unwin PN. 1985. Quaternary structure of the acetylcholine receptor. *Nature* 315:474–77
64. Unwin N. 2000. The Croonian Lecture 2000. Nicotinic acetylcholine receptor and the structural basis of fast synaptic transmission. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 355:1813–29
65. Changeux JP. 1969. Remarks on the symmetry and cooperative properties of biological membranes. In *Symmetry and Functions in Biological Systems at the Macromolecular Level*, ed. A Engström, B Strandberg, Nobel Symposium II. John Wiley and Sons Inc. New York, pp. 235–56
66. Hucho F, Changeux JP. 1973. Molecular weight and quaternary structure of the cholinergic receptor protein extracted by detergents from *Electrophorus electricus* electric tissue. *FEBS Lett.* 38:11–15
67. Weill CL, McNamee MG, Karlin A. 1974. Affinity-labeling of purified acetylcholine receptor from *Torpedo californica*. *Biochem. Biophys. Res. Commun.* 61:997–1003
68. Devillers-Thiery A, Changeux JP, Paroutaud P, Strosberg AD. 1979. The amino-terminal sequence of the 40000 molecular weight subunit of the acetylcholine receptor protein from *Torpedo marmorata*. *FEBS Lett.* 104:99–105
69. Raftery MA, Hunkapiller MW, Strader CD, Hood LE. 1980. Acetylcholine receptor: complex of homologous subunits. *Science* 208:1454–56
70. Noda M, Takahashi H, Tanabe T, Toyosato M, Furutani Y, et al. 1982. Primary structure of alpha-subunit precursor of *Torpedo californica* acetylcholine receptor deduced from cDNA sequence. *Nature* 299:793–97
71. Ballivet M, Patrick J, Lee J, Heinemann S. 1982. Molecular cloning of cDNA coding for the gamma subunit of *Torpedo* acetylcholine receptor. *Proc. Natl. Acad. Sci. USA* 79:4466–70
72. Giraudat J, Devillers-Thiéry A, Auffray C, Rougeon F, Changeux JP. 1982. Identification of a cDNA clone coding for the acetylcholine binding subunit of *Torpedo marmorata* acetylcholine receptor. *EMBO J.* 1:713–17
73. Devillers-Thiéry A, Giraudat J, Bentaboulet M, Changeux JP. 1983. Complete mRNA coding sequence of the acetylcholine binding alpha subunit of *Torpedo marmorata* acetylcholine receptor: a model for the transmembrane organization of the polypeptide chain. *Proc. Natl. Acad. Sci. USA* 80:2067–71

74. Kao PN, Dwork AJ, Kaldany RR, Silver ML, Wideman J, et al. 1984. Identification of the alpha subunit half-cystine specifically labeled by an affinity reagent for the acetylcholine receptor binding site. *J. Biol. Chem.* 259:11662–65
75. Oswald RE, Changeux JP. 1982. Crosslinking of alpha-bungarotoxin to the acetylcholine receptor from *Torpedo marmorata* by UV light irradiation. *FEBS Lett.* 139:225–29
76. Dennis M, Giraudat J, Kotzyba-Hibert F, Goeldner M, Hirth C, et al. 1988. Amino acids of the *Torpedo marmorata* acetylcholine receptor subunit labeled by a photoaffinity ligand for the acetylcholine binding site. *Biochemistry* 27:2346–57
77. Galzi JL, Revah F, Black D, Goeldner M, Hirth C, Changeux JP. 1990. Identification of a novel amino acid α-Tyr 93 within the active site of the acetylcholine receptor by photoaffinity labeling: additional evidence for a three-loop model of the acetylcholine binding site. *J. Biol. Chem.* 265:10430–37
78. Corringer PJ, Bertrand S, Bohler S, Edelstein SJ, Changeux JP, Bertrand D. 1998. Critical elements determining diversity in agonist binding and desensitization of neuronal nicotinic acetylcholine receptors. *J. Neurosci.* 18:648–57
79. Brejc K, van Dijk WJ, Klaassen RV, Schuurmans M, van Der Oost J, et al. 2001. Crystal structure of an ACh-binding protein reveals the ligand-binding domain of nicotinic receptors. *Nature* 411:269–76
80. Corringer PJ, Le Novère N, Changeux JP. 2000. Nicotinic receptors at the amino acid level. *Annu. Rev. Pharmacol. Toxicol.* 40:431–58
81. Changeux JP, Taly A. 2008. Nicotinic receptors, allosteric proteins and medicine. *Trends Mol. Med.* 14:93–102
82. Sixma TK. 2007. Nicotinic receptor structure emerging slowly. *Nat. Neurosci.* 10:937–38
83. Hansen SB, Sulzenbacher G, Huxford T, Marchot P, Taylor P, Bourne Y. 2005. Structures of Aplysia AChBP complexes with nicotinic agonists and antagonists reveal distinctive binding interfaces and conformations. *EMBO J.* 24:3635–46
84. Ihara M, Okajima T, Yamashita A, Oda T, Hirata K, et al. 2008. Crystal structures of *Lymnaea stagnalis* AChBP in complex with neonicotinoid insecticides imidacloprid and clothianidin. *Invert. Neurosci.* 8:71–81
85. Gotti C, Zoli M, Clementi F. 2006. Brain nicotinic acetylcholine receptors: native subtypes and their relevance. *Trends Pharmacol. Sci.* 27:482–91
86. Olsen RW, Sieghart W. 2008. International Union of Pharmacology. LXX. Subtypes of gamma-aminobutyric acid(A) receptors: classification on the basis of subunit composition, pharmacology and function. Update. *Pharmacol. Rev.* 60:24–60
87. Betz H, Laube B. 2006. Glycine receptors: recent insights into their structural organization and functional diversity. *J. Neurochem.* 97:1600–10
88. Barnes NM, Hales TG, Lummis SC, Peters JA. 2009. The 5-HT(3) receptor—the relationship between structure and function. *Neuropharmacology* 56:273–84
89. Changeux JP, Edelstein SJ. 2005. *Nicotinic Acetylcholine Receptors: from Molecular Biology to Cognition*. New York: Odile Jacob, 296 pp.
90. Weber M, Changeux JP. 1974. Binding of *Naja nigricollis* 3H-alpha-toxin to membrane fragments from *Eletrophorus* and *Torpedo* electric organs. 3. Effect of local anaesthetics on the binding of the tritiated-neurotoxin. *Mol. Pharmacol.* 10:35–40
91. Cohen JB, Weber M, Changeux JP. 1974. Effects of local anesthetics and calcium on the interaction of cholinergic ligands with the nicotinic receptor protein from *Torpedo marmorata*. *Mol. Pharmacol.* 10:904–32
92. Sobel A, Weber M, Changeux JP. 1977. Large-scale purification of the acetylcholine-receptor protein in its membrane-bound and detergent-extracted forms from *Torpedo marmorata* electric organ. *Eur. J. Biochem.* 80:215–24
93. Oswald RE, Changeux JP. 1981. Selective labeling of the delta-subunit of the acetylcholine receptor by a covalent local anesthetic. *Biochemistry* 20:7166–74
94. Oswald R, Changeux JP. 1981. Ultraviolet light-induced labeling by noncompetitive blockers of the acetylcholine receptor from *Torpedo marmorata*. *Proc. Natl. Acad. Sci. USA* 78:3925–29

95. Heidmann T, Oswald R, Changeux JP. 1982. The high affinity binding site for chlorpromazine is present only as a single copy per cholinergic receptor molecule and is shared by four polypeptide chains. *C. R. Seances Acad. Sci. III* 295:345–49
96. Heidmann T, Bernhardt J, Neumann E, Changeux JP. 1983. Rapid kinetics of agonist binding and permeability response analyzed in parallel on acetylcholine receptor-rich membranes from *Torpedo marmorata*. *Biochemistry* 22:5452–59
97. Heidmann T, Changeux JP. 1984. Time-resolved photolabeling by the noncompetitive blocker chlorpromazine of the acetylcholine receptor in its transiently open and closed ion channel conformations. *Proc. Natl. Acad. Sci. USA* 81:1897–901
98. Heidmann T, Changeux JP. 1986. Characterization of the transient agonist-triggered state of the acetylcholine receptor rapidly labeled by the noncompetitive blocker [3H]chlorpromazine: additional evidence for the open channel conformation. *Biochemistry* 25:6109–13
99. Giraudat J, Dennis M, Heidmann T, Chang JY, Changeux JP. 1986. Structure of the high affinity site for noncompetitive blockers of the acetylcholine receptor: serine-262 of the delta subunit is labeled by [3H]-chlorpromazine. *Proc. Natl. Acad. Sci. USA* 83:2719–23
99a. Oberthür W, Muhn P, Baumann H, Lottspeich F, Wittmann-Liebold B, Hucho F. 1986. The reaction site of a noncompetitive antagonist in the delta subunit of the nicotinic acetylcholine receptor. *EMBO J.* 5:1815–19
100. Giraudat J, Dennis M, Heidmann T, Haumont PY, Lederer F, Changeux JP. 1987. Structure of the high-affinity binding site for noncompetitive blockers of the acetylcholine receptor: [3H] chlorpromazine labels homologous residues in the beta and delta chains. *Biochemistry* 26:2410–18
101. Revah F, Galzi JL, Giraudat J, Haumont PY, Lederer F, Changeux JP. 1990. The noncompetitive blocker [3H]-chlorpromazine labels three amino acids of the acetylcholine receptor gamma subunit: implications for the alpha-helical organization of the M2 segments and the structure of the ion channel. *Proc. Natl. Acad. Sci. USA* 87:4675–79
102. Barnard EA, Miledi R, Sumikawa K. 1982. Translation of exogenous messenger RNA coding for nicotinic acetylcholine receptors produces functional receptors in *Xenopus* oocytes. *Proc. R. Soc. Lond. B Biol. Sci.* 215:241–46
103. Imoto K, Methfessel C, Sakmann B, Mishina M, Mori Y, et al. 1986. Location of a delta-subunit region determining ion transport through the acetylcholine receptor channel. *Nature* 324:670–74
104. Imoto K, Busch C, Sakmann B, Mishina M, Konno T, et al. 1988. Rings of negatively charged amino acids determine the acetylcholine receptor channel conductance. *Nature* 335:645–48
105. Leonard RJ, Labarca CG, Charnet P, Davidson N, Lester HA. 1988. Evidence that the M2 membrane-spanning region lines the ion channel pore of the nicotinic receptor. *Science* 242:1578–81
106. Galzi JL, Devillers-Thiery A, Hussy N, Bertrand S, Changeux JP, Bertrand D. 1992. Mutations in the ion channel domain of a neuronal nicotinic receptor convert ion selectivity from cationic to anionic. *Nature* 359:500–5
107. Corringer PJ, Bertrand S, Galzi JL, Devillers-Thiéry A, Changeux JP, Bertrand D. 1999. Mutational analysis of the charge selectivity filter of the alpha7 nicotinic acetylcholine receptor. *Neuron* 22:831–43
108. Wotring VE, Weiss DS. 2008. Charge scan reveals an extended region at the intracellular end of the GABA receptor pore that can influence ion selectivity. *J. Gen. Physiol.* 131:87–97
109. Keramidas A, Moorhouse AJ, Schofield PR, Barry PH. 2004. Ligand-gated ion channels: mechanisms underlying ion selectivity. *Prog. Biophys. Mol. Biol.* 86:161–204
110. Sunesen M, Prado de Carvalho L, Dufresne V, Grailhe R, Savatier-Duclert N, et al. 2006. Mechanism of Cl selection by a GluCl receptor revealed through mutations in the selectivity filter. *J. Biol. Chem.* 281:14875–81
111. Gunthorpe MJ, Lummis SC. 2001. Conversion of the ion selectivity of the 5-HT(3a) receptor from cationic to anionic reveals a conserved feature of the ligand-gated ion channel superfamily. *J. Biol. Chem.* 276:10977–83
112. Eiselé JL, Bertrand S, Galzi JL, Devillers-Thiéry A, Changeux JP, Bertrand D. 1993. Chimeric nicotinic-serotonergic receptor combines distinct ligand binding and channel specificities. *Nature* 366:479–83
113. Bocquet N, Nury H, Baaden M, Le Poupon C, Changeux JP, et al. 2009. X-ray structure of a pentameric ligand-gated ion channel in an apparently open conformation. *Nature* 457:111–14

114. Hilf RJ, Dutzler R. 2009. Structure of a potentially open state of a proton-activated pentameric ligand-gated ion channel. *Nature* 457:115–18
115. Valeur B, Patrick J, Podleski T, Monnerie L, Changeux JP. 1970. Perfecting an apparatus allowing to measure changes of extrinsic fluorescence intensity associated with electric excitation of electroplaque isolated from gymnotus. *C. R. Acad. Sci. D* 270:2583–86
116. Patrick J, Valeur B, Monnerie L, Changeux JP. 1971. Changes in extrinsic fluorescence intensity of the electroplax membrane during electrical excitation. *J. Memb. Biol.* 5:102–20
117. Kasai M, Changeux JP, Monnerie L. 1969. In vitro interaction of 1-anilino 8 naphthalene sulfonate with excitable membranes isolated from the electric organ of *Electrophorus electricus*. *Biochem. Biophys. Res. Commun.* 36:420–27
118. Wahl P, Kasai M, Changeux JP. 1971. A study on the motion of proteins in excitable membrane fragments by nanosecond fluorescence polarization spectroscopy. *Eur. J. Biochem.* 18:332–41
119. Katz B, Thesleff S. 1957. A study of the desensitization produced by acetylcholine at the motor end-plate. *J. Physiol.* 138:63–80
120. Weber M, David-Pfeuty MT, Changeux JP. 1975. Regulation of binding properties of the nicotinic receptor protein by cholinergic ligands in membrane fragments from *Torpedo marmorata*. *Proc. Natl. Acad. Sci. USA* 72:3443–47
121. Colquhoun D, Rang HP. 1976. Effects of inhibitors of the binding of iodinated alpha-bungarotoxin to acetylcholine receptors in rat muscle. *Mol. Pharmacol.* 12:519–35
122. Weiland G, Georgia B, Lappi S, Chignell CF, Taylor P. 1977. Kinetics of agonist-mediated transitions in state of the cholinergic receptor. *J. Biol. Chem.* 252:7648–56
123. Grünhagen HH, Changeux JP. 1976. Studies on the electrogenic action of acetylcholine with *Torpedo marmorata* electric organ. IV. Quinacrine: a fluorescent probe for the conformational transitions of the cholinergic receptor protein in its membrane bound state. *J. Mol. Biol.* 106:497–516. V. Qualitative correlation between pharmacological effects and equilibration processes of the cholinergic receptor protein as revealed by the structural probe quinacrine. *J. Mol. Biol.* 106:517–35
124. Grünhagen HH, Iwatsubo M, Changeux JP. 1977. Fast kinetic studies on the interaction of cholinergic agonists with the membrane-bound acetylcholine receptor from *Torpedo marmorata* as revealed by quinacrine fluorescence. *Eur. J. Biochem.* 80:225–42
125. Heidmann T, Changeux JP. 1979. Fast kinetic studies on the interaction of a fluorescent agonist with the membrane-bound acetylcholine receptor from *Torpedo marmorata*. *Eur. J. Biochem.* 94:255–79; Id. Fast kinetic studies on the allosteric interactions between acetylcholine receptor and local anesthetic binding sites. *Eur. J. Biochem.* 94:281–96
126. Heidmann T, Changeux JP. 1980. Interaction of a fluorescent agonist with the membrane-bound acetylcholine receptor from *Torpedo marmorata* in the millisecond time range: resolution of an "intermediate" conformational transition and evidence for positive cooperative effects. *Biochem. Biophys. Res. Comm.* 97:889–96
127. Jackson MB. 1984. Spontaneous openings of the acetylcholine receptor channel. *Proc. Natl. Acad. Sci. USA* 81:3901–4
128. Purohit P, Auerbach A. 2009. Unliganded gating of acetylcholine receptor channels. *Proc. Natl. Acad. Sci. USA* 106:115–20
129. Edelstein S, Schaad O, Henry E, Bertrand D Changeux JP. 1996. A kinetic mechanism for nicotinic acetylcholine receptors based on multiple allosteric transitions. *Biol. Cybern.* 75:361–79
130. Miyazawa A, Fujiyoshi Y, Unwin N. 2003. Structure and gating mechanism of the acetylcholine receptor pore. *Nature* 423:949–55
131. Paas Y, Gibor G, Grailhe R, Savatier-Duclert N, Dufresne V, et al. 2005. Pore conformations and gating mechanism of a Cys-loop receptor. *Proc. Natl. Acad. Sci. USA* 102:15877–82
132. Le Novère N, Corringer PJ, Changeux JP. 1999. Improved secondary structure predictions for a nicotinic receptor subunit. Incorporation of solvent accessibility and experimental data into a 2D representation. *Biophys. J.* 76:2329–45
133. Le Novère N, Grutter T, Changeux JP. 2002. Models of the extracellular domain of the nicotinic receptors and of agonist- and Ca^{2+}-binding sites. *Proc. Natl. Acad. Sci. USA* 99:3210–15

134. Fruchart-Gaillard C, Gilquin B, Antil-Delbeke S, Le Novère N, Tamiya T, et al. 2002. Experimentally-based model of a complex between a snake toxin and the alpha 7 nicotinic receptor. *Proc. Natl. Acad. Sci. USA* 99:3216–21
135. Taly A, Delarue M, Grutter T, Nilges M, Le Novère N, et al. 2005. Normal mode analysis suggests a quaternary twist model for the nicotinic receptor gating mechanism. *Biophys. J.* 88:3954–65
136. Cheng X, Lu B, Grant B, Law RJ, McCammon JA. 2006. Channel opening motion of alpha 7 nicotinic acetylcholine receptor as suggested by normal mode analysis. *J. Mol. Biol.* 355:310–24
137. Samson AO, Levitt M. 2008. Inhibition mechanism of the acetylcholine receptor by alpha-neurotoxins as revealed by normal-mode dynamics. *Biochemistry* 47:4065–70
137a. Tasneem A, Iyer LM, Jakobsson E, Aravind L. 2005. Identification of the prokaryotic ligand-gated ion channels and their implications for the mechanisms and origins of animal Cys-loop ion channels. *Genome Biol.* 6(1):R4
138. Bocquet N, Prado de Carvalho L, Cartaud J, Neyton J, Le Poupon C, et al. 2007. A prokaryotic proton-gated ion channel from the nicotinic acetylcholine receptor family. *Nature* 445:116–19
139. Bocquet N, Nury H, Baaden M, Le Poupon C, Changeux JP, et al. X-ray structure of a pentameric ligand-gated ion channel in an apparently open conformation. *Nature* 457:111–14
139a. Hilf RJ, Dutzler R. 2009. Structure of a potentially open state of a proton-activated pentameric ligand-gated ion channel. *Nature* 457:115–18
140. Hilf RJ, Dutzler R. 2008. X-ray structure of a prokaryotic pentameric ligand-gated ion channel. *Nature* 452:375–79
141. Couturier S, Bertrand D, Matter JM, Hernandez MC, Bertrand S, et al. 1990. A neuronal nicotinic acetylcholine receptor subunit (alpha 7) is developmentally regulated and forms a homo-oligomeric channel blocked by alpha-BTX. *Neuron* 5:847–56
142. Couturier S, Erkman L, Valera S, Rungger D, Bertrand S, et al. 1990. Alpha 5, alpha 3, and non-alpha 3. Three clustered avian genes encoding neuronal nicotinic acetylcholine receptor-related subunits. *J. Biol. Chem.* 265:17560–67
143. Revah F, Bertrand D, Galzi JL, Devillers-Thiéry A, Mulle C, et al. 1991. Mutations in the channel domain alter desensitization of a neuronal nicotinic receptor. *Nature* 353:846–49
144. Bertrand D, Devillers-Thiery A, Revah F, Galzi JL, Hussy N, et al. 1992. Unconventional pharmacology of a neuronal nicotinic receptor mutated in the channel domain. *Proc. Natl. Acad. Sci. USA* 89:1261–65
145. Engel AG, Ohno K. 2002. Congenital myasthenic syndromes. *Adv. Neurol.* 88:203–15
146. Edelstein S, Schaad O, Changeux JP. 1997. Myasthenic nicotinic receptor mutant interpreted in terms of the allosteric model. *C. R. Acad. Sci. Paris* 320:953–61
147. Steinlein OK, Mulley JC, Propping P, Wallace RH, Phillips HA, et al. 1995. A missense mutation in the neuronal nicotinic acetylcholine receptor alpha 4 subunit is associated with autosomal dominant nocturnal frontal lobe epilepsy. *Nat. Genet.* 11:201–3
148. Taly A, Corringer PJ, Grutter T, Prado de Carvalho L, Karplus M, Changeux JP. 2006. Implications of the quaternary twist allosteric model for the physiology and pathology of nicotinic acetylcholine receptors. *Proc. Natl. Acad. Sci. USA* 103:16965–70
149. Lefkowitz RJ, Cotecchia S, Samama P, Costa T. 1993. Constitutive activity of receptors coupled to guanine nucleotide regulatory proteins. *Trends Pharmacol. Sci.* 14:303–7
150. Changeux JP. 1981. The acetylcholine receptor: an "allosteric" membrane protein. *Harvey Lect.* 75:85–254
151. Mulle C, Léna C, Changeux JP. 1992. Potentiation of nicotinic receptor response by external calcium in rat central neurons. *Neuron* 8:937–45
152. Galzi JL, Bertrand S, Corringer PJ, Changeux JP, Bertrand D. 1996. Identification of calcium binding sites that regulate potentiation of a neuronal nicotinic acetylcholine receptor. *EMBO J.* 15:5824–32
153. Krause RM, Buisson B, Bertrand S, Corringer PJ, Galzi JL, et al. 1998. Ivermectin: a positive allosteric effector of the alpha 7 neuronal nicotinic acetylcholine receptor. *Mol. Pharmacol.* 53:283–94
154. Young GT, Zwart R, Walker AS, Sher E, Millar NS. 2008. Potentiation of alpha 7 nicotinic acetylcholine receptors via an allosteric transmembrane site. *Proc. Natl. Acad. Sci. USA* 105:14686–91

154a. Bertrand D, Bertrand S, Cassar S, Gubbins E, Li J, Gopalakrishnan M. 2008. Positive allosteric modulation of the alpha7 nicotinic acetylcholine receptor: ligand interactions with distinct binding sites and evidence for a prominent role of the M2-M3 segment. *Mol. Pharmacol.* 74(5):1407–16
155. Popot JL, Demel RA, Sobel A, Van Deenen LL, Changeux JP. 1978. Interaction of the acetylcholine (nicotinic) receptor protein from *Torpedo marmorata* electric organ with monolayers of pure lipids. *Eur. J. Biochem.* 85:27–42
156. Li GD, Chiara DC, Sawyer GW, Husain SS, Olsen RW, Cohen JB. 2006. Identification of a GABAA receptor anesthetic binding site at subunit interfaces by photolabeling with an etomidate analog. *J Neurosci.* 26:11599–605
157. Teichberg VI, Sobel A, Changeux JP. 1977. In vitro phosphorylation of acetylcholine receptor. *Nature* 267:540–42
158. Fontaine B, Klarsfeld A, Hökfelt T, Changeux JP. 1986. Calcitonin gene-related peptide, a peptide present in spinal cord motoneurons, increases the number of acetylcholine receptors in primary cultures of chick embryo myotubes. *Neurosci. Lett.* 71:59–65
159. Mulle C, Benoit P, Pinset C, Roa M, Changeux JP. 1988. Calcitonin gene-related peptide enhances the rate of desensitization of the nicotinic acetylcholine receptor in cultured mouse muscle cells. *Proc. Natl. Acad. Sci. USA* 85:5728–32
160. Marks MJ, Burch JB, Collins AC. 1983. Effects of chronic nicotine infusion on tolerance development and nicotinic receptors. *J. Pharmacol. Exp. Ther.* 226:817–25
161. Schwartz RD, Kellar KJ. 1985. In vivo regulation of [3H]acetylcholine recognition sites in brain by nicotinic cholinergic drugs. *J. Neurochem.* 45:427–33
162. Sallette J, Bohler S, Benoit P, Soudant M, Pons S, et al. 2004. An extracellular protein microdomain controls up-regulation of neuronal nicotinic acetylcholine receptors by nicotine. *J. Biol. Chem.* 279:18767–75
163. Sallette J, Pons S, Devillers-Thiery A, Soudant M, Prado de Carvalho L, et al. 2005. Nicotine upregulates its own receptors through enhanced intracellular maturation. *Neuron* 46:595–607
164. Heidmann T, Changeux JP. 1982. Molecular model of the regulation of chemical synapse efficiency at the postsynaptic level. *C. R. Seances Acad. Sci. III* 295:665–70
165. Dehaene S, Changeux JP, Nadal JP. 1987. Neural networks that learn temporal sequences by selection. *Proc. Natl. Acad. Sci. USA* 84:2727–31
166. Dehaene S, Changeux JP. 1989. A simple model of prefrontal cortex function in delayed-response tasks. *J. Cognitive Neurosci.* 1:244–61
167. Dehaene S, Changeux JP. 1991. The Wisconsin Card Sorting Test: theoretical analysis and simulation of a reasoning task in a model neuronal network. *Cereb. Cortex* 1:62–79
168. Hanson SM, Czajkowski C. 2008. Structural mechanisms underlying benzodiazepine modulation of the GABA(A) receptor. *J. Neurosci.* 28:3490–99
169. Olsen RW, Chang CS, Li G, Hanchar HJ, Wallner M. 2004. Fishing for allosteric sites on GABA(A) receptors. *Biochem. Pharmacol.* 68:1675–84
170. Wiesel TN, Hubel DH. 1965. Extent of recovery from the effects of visual deprivation in kittens. *J Neurophysiol.* 28:1060–72
171. Changeux JP. 1972. Le cerveau et l'évènement. *Communications Ecole des Hautes Etudes en Sciences Sociales.* 18–37
172. Changeux JP, Courrège P, Danchin A. 1973. A theory of the epigenesis of neural networks by selective stabilization of synapses. *Proc. Natl. Acad. Sci. USA* 70:2974–78
173. Changeux JP, Danchin A. 1976. Selective stabilization of developing synapses as a mechanism for the specificication of neuronal networks. *Nature* 264:705–12
174. Changeux JP. 2005. *The Physiology of Truth*. 324 pp. Cambridge, MA: Harvard Univ. Press
175. Heidmann A, Heidmann T, Changeux JP. 1984. Selective stabilization of neuronal representations by resonance between spontaneous prerepresentations of the cerebral network and percepts evoked by interaction with the outside world. *C. R. Acad. Sci. III* 299:839–44
176. Dehaene S, Changeux JP. 1997. A hierarchical neuronal network for planning behavior. *Proc. Natl. Acad. Sci. USA* 94:13293–98

177. Barto AG, Sutton RS, Anderson CW. 1983. Neuron like elements that can solve difficult learning control problems. *IEEE Trans. Syst. Man Cyber. SMC* 13:834–46
178. Friston KJ, Tononi G, Reeke GN Jr, Sporns O, Edelman GM. 1994. Value-dependent selection in the brain: simulation in a synthetic neural model. *Neuroscience* 59:229–43
179. Montague PR, Dayan P, Sejnowski TJ. 1996. A framework for mesencephalic dopamine systems based on predictive Hebbian learning. *J. Neurosci.* 16:1936–47
180. Schultz W, Dayan P, Montague PR. 1997. A neural substrate of prediction and reward. *Science* 275:1593–99
181. Crick F, Koch C. 1990. Some reflections on visual awareness. *Cold Spring Harb. Symp. Quant. Biol.* 55:953–62
182. Llinás RR, Paré D. 1991. Of dreaming and wakefulness. *Neuroscience* 44:521–35
183. Baars BJ. 1989. *A Cognitive Theory of Consciousness*. Cambridge, MA: Cambridge Univ. Press
184. Dehaene S, Kerszberg M, Changeux JP. 1998. A neuronal model of a global workspace in effortful cognitive tasks. *Proc. Natl. Acad. Sci. USA* 95:14529–34
185. Dehaene S, Sergent C, Changeux JP. 2003. A neuronal network model linking subjective reports and objective physiological data during conscious perception. *Proc. Natl. Acad. Sci. USA* 100:8520–25
186. Dehaene S, Changeux JP. 2005. Ongoing spontaneous activity controls access to consciousness: a neuronal model for inattentional blindness. *PLoS Biol.* 3:e141
187. Del Cul A, Baillet S, Dehaene S. 2007. Brain dynamics underlying the nonlinear threshold for access to consciousness. *PLoS Biol.* 5:e260
188. Bekinschtein TA, Dehaene S, Rohaut B, Tadel F, Cohen L, Naccache L. 2009. Neural signature of the conscious processing of auditory regularities. *Proc. Natl. Acad. Sci. USA* 106:1672–77
189. Redfern PA. 1970. Neuromuscular transmission in new-born rats. *J. Physiol.* 209:701–9
190. Benoit P, Changeux JP. 1975. Consequences of tenotomy on the evolution of multiinnervation in developing rat soleus muscle. *Brain Res.* 99:354–58
191. Benoit P, Changeux JP. 1978. Consequences of blocking the nerve with a local anesthetic on the evolution of multiinnervation at the regenerating neuromuscular junction of the rat. *Brain Res.* 149:89–96
192. Crepel F, Mariani J, Korn H, Changeux JP. 1974. Electrophysiology of the cerebellar cortex in the staggerer mutant mouse. *C. R. Acad. Sci. Hebd. Seances Acad. Sci. D* 277:2761–63
193. Mariani J, Changeux JP. 1981. Ontogenesis of olivocerebellar relationships. I. Studies by intracellular recordings of the multiple innervation of Purkinje cells by climbing fibers in the developing rat cerebellum. *J. Neurosci.* 1:696–702; II. Spontaneous activity of inferior olivary neurons and climbing fiber mediated activity of cerebellar Purkinje cells in developing rats. *J. Neurosci.* 1:703–9
194. Benoit P, Delhaye-Bouchaud N, Changeux JP, Mariani J. 1984. Stability of multiple innervation of Purkinje cells by climbing fibers in the agranular cerebellum of old rats X-irradiated at birth. *Brain Res.* 316:310–13
195. Rossi FM, Pizzorusso T, Porciatti V, Marubio LM, Maffei L, Changeux JP. 2001. Requirement of the nicotinic acetylcholine receptor beta 2 subunit for the anatomical and functional development of the visual system. *Proc. Natl. Acad. Sci. USA*. 98:6453–58
196. Grubb MS, Rossi FM, Changeux JP, Thompson ID. 2003. Abnormal functional organization in the dorsal lateral geniculate nucleus of mice lacking the beta 2 subunit of the nicotinic acetylcholine receptor. *Neuron* 40:1161–72
197. Lichtman JW, Colman H. 2000. Synapse elimination and indelible memory. *Neuron* 25:269–78
198. Luo L, O'Leary DD. 2005. Axon retraction and degeneration in development and disease. *Annu. Rev. Neurosci.* 28:127–56
199. Karlsgodt KH, Sun D, Jimenez AM, Lutkenhoff ES, Willhite R, et al. 2008. Developmental disruptions in neural connectivity in the pathophysiology of schizophrenia. *Dev. Psychopathol. Fall.* 20:1297–327
200. Bourgeois JP, Ryter A, Menez A, Fromageot P, Boquet P, Changeux JP. 1972. Localization of the cholinergic receptor protein in *Electrophorus* electroplax by high resolution autoradiography. *FEBS Lett.* 25:127–33

201. Bourgeois JP, Popot JL, Ryter A, Changeux JP. 1978. Quantitative studies on the localization of the cholinergic receptor protein in the normal and denervated electroplaque from *Electrophorus electricus*. *J. Cell Biol.* 79:200–16
202. Merlie JP, Sobel A, Changeux JP, Gros F. 1975. Synthesis of acetylcholine receptor during differentiation of cultured embryonic muscle cells. *Proc. Natl. Acad. Sci. USA* 72:4028–32
203. Merlie JP, Changeux JP, Gros F. 1978. Skeletal muscle acetylcholine receptor. Purification, characterization, and turnover in muscle cell cultures. *J. Biol. Chem.* 253:2882–91
204. Betz H, Bourgeois JP, Changeux JP. 1977. Evidence for degradation of the acetylcholine (nicotinic) receptor in skeletal muscle during the development of the chick embryo. *FEBS Lett.* 77:219–24
205. Bourgeois JP, Betz H, Changeux JP. 1978. Effects of chronic paralysis of chick embryo by flaxedil on the development of the neuromuscular junction. *C. R. Acad. Sci. Hebd. Seances Acad. Sci. D* 286:773–76
206. Merlie J, Sanes JR. 1985. Concentration of acetylcholine receptor mRNA in synaptic regions of adult muscle fibres. *Nature* 317:66–68
207. Fontaine B, Sassoon D, Buckingham M, Changeux JP. 1988. Detection of the nicotinic acetylcholine receptor alpha-subunit mRNA by in situ hybridization at neuromuscular junctions of 15-day old chick striated muscles. *EMBO J.* 7:603–9
208. Fontaine B, Changeux JP. 1989. Localization of nicotinic acetylcholine receptor alpha-subunit transcripts during myogenesis and motor endplate development in the chick. *J. Cell Biol.* 108:1025–37
209. Piette J, Bessereau JL, Huchet M, Changeux JP. 1990. Two adjacent MyoD1-binding sites regulate expression of the acetylcholine receptor alpha-subunit gene. *Nature* 345:353–55
210. Bessereau JL, Stratford-Perricaudet LD, Piette J, Le Poupon C, Changeux JP. 1994. In vivo and in vitro analysis of electrical activity-dependent expression of muscle acetylcholine receptor genes using adenovirus. *Proc. Natl. Acad. Sci. USA* 91:1304–8
211. Koike S, Schaeffer L, Changeux JP. 1995. Identification of a DNA element determining synaptic expression of the mouse acetylcholine receptor delta-subunit gene. *Proc. Natl. Acad. Sci. USA* 92:10624–28
212. Schaeffer L, Duclert N, Huchet-Dymanus M, Changeux JP. 1998. Implication of a multisubunit Ets-related transcription factor in synaptic expression of the nicotinic acetylcholine receptor. *EMBO J.* 17:3078–90
213. Jasmin BJ, Cartaud J, Bornens M, Changeux JP. 1989. Golgi apparatus in chick skeletal muscle: changes in its distribution during end plate development and after denervation. *Proc. Natl. Acad. Sci. USA* 86:7218–22
214. Jasmin BJ, Changeux JP, Cartaud J. 1990. Compartmentalization of cold-stable and acetylated microtubules in the subsynaptic domain of chick skeletal muscle fiber. *Nature* 344:673–75
215. Zoli M, Le Novère N, Hill J, Changeux JP. 1995. Developmental regulation of nicotinic receptor subunit mRNAs in the rat central and peripheral nervous system. *J. Neurosci.* 15:1912–39
216. Bessis A, Savatier N, Devillers-Thiery A, Bejanin S, Changeux JP. 1993. Negative regulatory elements upstream of a novel exon of the neuronal nicotinic acetylcholine receptor a-2 subunit gene. *Nucleic Acids Res.* 21:2185–92
217. Bessis A, Champtiaux N, Chatelin L, Changeux JP. 1997. The neuron-restrictive silencer element: a dual enhancer/silencer crucial for patterned expression of a nicotinic receptor gene in the brain. *Proc. Natl. Acad. Sci. USA* 94:5906–11
218. Mulle C, Changeux JP. 1990. A novel type of nicotinic receptor in the rat central nervous system characterized by patch-clamp techniques. *J. Neurosci.* 10:169–75
219. Mulle C, Vidal C, Benoit P, Changeux JP. 1991. Existence of different subtypes of nicotinic acetylcholine receptors in the rat habenulo interpeduncular system. *J. Neurosci.* 11:2588–97
220. Léna C, Changeux JP, Mulle C. 1993. Evidence for "preterminal" nicotinic receptors on GABAergic axons in the rat interpeduncular nucleus. *J. Neurosci.* 13:2680–88
221. McGehee DS, Role LW. 1996. Presynaptic ionotropic receptors. *Curr. Opin. Neurobiol.* 6:342–49
222. Kawai H, Lazar R, Metherate R. 2007. Nicotinic control of axon excitability regulates thalamocortical transmission. *Nat. Neurosci.* 10:1168–75
223. Vidal C, Changeux JP. 1989. Pharmacological profile of nicotinic acetylcholine receptors in the rat prefrontal cortex: an electrophysiological study in a slice preparation. *Neuroscience* 29:261–70

224. Vidal C, Changeux JP. 1993. Nicotinic and muscarinic modulations of excitatory synaptic transmission in the rat prefrontal cortex in vitro. *Neuroscience* 56:23–32
225. Léna C, de Kerchove d'Exaerde A, Cordero-Erausquin M, Le Novère N, Arroyo-Jimenez MM, Changeux JP. 1999. Diversity and distribution of nicotinic acetylcholine receptors in the *locus ceruleus* neurons. *Proc. Natl. Acad. Sci. USA* 96:12127–31
226. Klink R, de Kerchove d'Exaerde A, Zoli M, Changeux JP. 2001. Molecular and physiological diversity of nicotinic acetylcholine receptors in the midbrain dopaminergic nuclei. *J. Neurosci.* 21:1452–63
227. Maggi L, Le Magueresse C, Changeux JP, Cherubini E. 2003. Nicotine activates immature "silent" connections in the developing hippocampus. *Proc. Natl. Acad. Sci. USA* 100:2059–64
228. Levin ED. 1992. Nicotinic systems and cognitive function. *Psychopharmacology (Berlin)* 108:417–31
229. Nordberg A, Winblad B. 1986. Reduced number of [3H]nicotine and [3H]acetylcholine binding sites in the frontal cortex of Alzheimer brains. *Neurosci. Lett.* 72:115–19
230. Granon S, Vidal C, Thinus-Blanc C, Changeux JP, Poucet B. 1994. Working memory, response selection and effortful processing in rats with medial, prefrontal lesions. *Behav. Neurosci.* 108:883–91
231. Granon S, Poucet B, Thinus-Blanc C, Changeux JP, Vidal C. 1995. Nicotinic and muscarinic receptors in the rat prefrontal cortex: differential roles in working memory, response selection, and effortful processing. *Psychopharmacology* 119:139–44
232. Picciotto M, Zoli M, Léna C, Bessis A, Lallemand Y, et al. 1995. Abnormal avoidance learning in mice lacking functional high-affinity nicotine receptor in the brain. *Nature* 374:65–67
233. Picciotto MR, Zoli M, Rimondini R, Léna C, Marubio L, et al. 1998. Acetylcholine receptors containing the beta2-subunit are involved in the reinforcing properties of nicotine. *Nature* 391:173–77
234. Marubio LM, del Mar Arroyo-Jimenez M, Cordero-Erausquin M, Léna C, Le Novère N, et al. 1999. Reduced antinociception in mice lacking neuronal nicotinic receptor subunits. *Nature* 398:805–10
235. Champtiaux N, Gotti C, Cordero-Erausquin M, David DJ, Przybylski C, et al. 2003. Subunit composition of functional nicotinic receptors in dopaminergic neurons investigated with knock-out mice. *J. Neurosci.* 23:7820–29
236. Granon S, Faure P, Changeux JP. 2003. Executive and social behaviors under nicotinic receptor regulation. *Proc. Natl. Acad. Sci. USA* 100:9596–601
236a. Zoli M, Picciotto M, Ferrari R, Cocchi D, Changeux J-P. 1999. Increased neurodegeneration during ageing in mice lacking high-affinity nicotine receptors. *EMBO J.* 18:1235–44
237. Maskos U, Molles BE, Pons S, Besson M, Guiard BP, et al. 2005. Nicotine reinforcement and cognition restored by targeted expression of nicotinic receptors. *Nature* 436:103–7
238. Mameli-Engvall M, Evrard A, Pons S, Maskos U, Svensson TH, et al. 2006. Hierarchical control of dopamine neuron-firing patterns by nicotinic receptors. *Neuron* 50:911–21
239. Avale ME, Faure P, Pons S, Robledo P, Deltheil T, et al. 2008. Interplay of beta2* nicotinic receptors and dopamine pathways in the control of spontaneous locomotion. *Proc. Natl. Acad. Sci. USA* 105:15991–96
240. Andres C. 2002. Molecular genetics and animal models in autistic disorder. *Brain Res. Bull.* 57:109–19
241. Naldini L, Blömer U, Gauge FH, Trono D, Verma IM. 1996. Efficient transfer, integration, and sustained long-term expression of the transgene in adult rat brains injected with a lentiviral vector. *Proc. Natl. Acad. Sci. USA* 93:11382–88
242. Janhunen S, Ahtee L. 2007. Differential nicotinic regulation of the nigrostriatal and mesolimbic dopaminergic pathways: implications for drug development. *Neurosci. Biobehav. Rev.* 31:287–314
243. Pons S, Fattore L, Cossu G, Tolu S, Porcu E, et al. 2008. Crucial role of alpha4 and alpha6 nicotinic acetylcholine receptor subunits from ventral tegmental area in systemic nicotine self-administration. *J. Neurosci.* 28:12318–27
244. Besson VM, Granon S, Mameli-Engvall M, Cloëz-Tayarani I, Maubourguet N, et al. 2007. Long-term effects of chronic nicotine exposure on brain nicotinic receptors. *Proc. Natl. Acad. Sci. USA* 104:8155–60
245. Changeux JP, Dehaene S. 2008. The Neuronal Workspace Model: conscious processing and learning. In *Learning Theory and Behavior*, ed. R Menzel, Vol. 1 of Learning and Memory: A Comprehensive Reference. J. Byrne, editor, pp. 729–758. Oxford: Elsevier
246. Changeux JP. 2006. The Ferrier Lecture 1998. The molecular biology of consciousness investigated with genetically modified mice. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 361:2239–59

247. Gutkin BS, Dehaene S, Changeux JP. 2006. A neurocomputational hypothesis for nicotine addiction. *Proc. Natl. Acad. Sci. USA* 103:1106–11
248. Musso F, Bettermann F, Vucurevic G, Stoeter P, Konrad A, Winterer G. 2007. Smoking impacts on prefrontal attentional network function in young adult brains. *Psychopharmacology (Berl.)* 191:159–69
249. Cohen G, Han ZY, Grailhe R, Gallego J, Gaultier C, et al. 2002. beta 2 nicotinic acetylcholine receptor subunit modulates protective responses to stress: a receptor basis for sleep-disordered breathing after nicotine exposure. *Proc. Natl. Acad. Sci. USA* 99:13272–77
250. Cohen G, Roux JC, Grailhe R, Malcolm G, Changeux JP, Lagercrantz H. 2005. Perinatal exposure to nicotine causes deficits associated with a loss of nicotinic receptor function. *Proc. Natl. Acad. Sci. USA* 102:3817–21
251. Wiklund A, Granon S, Cloëz-Tayarani I, Faure P, le Sourd AM, et al. 2005. Sevoflurane anesthesia alters exploratory and anxiety-like behavior in mice lacking the beta2 nicotinic acetylcholine receptor subunit. *Anesthesiology* 109:790–98
252. Taly A, Corringer P-J, Guedin D, Lestage P, Changeux J-P. 2009. Nicotinic receptors: allosteric transitions and therapeutic targets in the nervous system. *Nat. Rev. Drug Discovery* 8:733–50