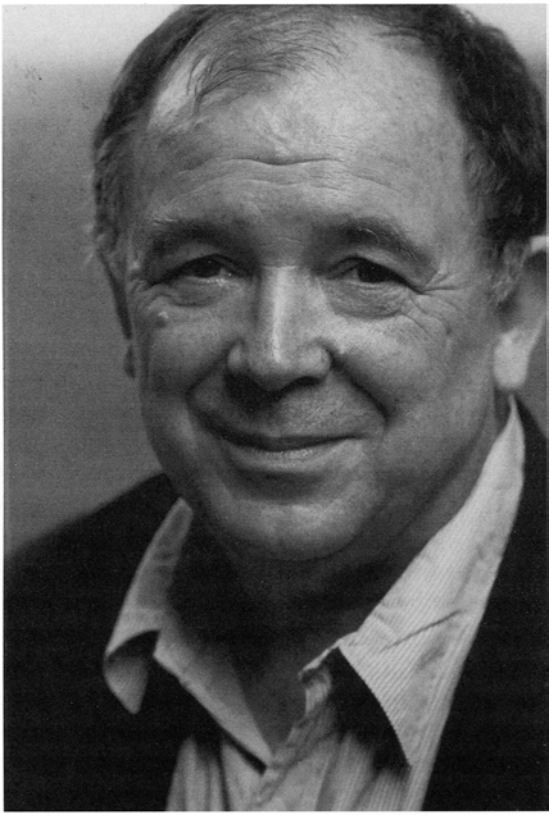
The History of Neuroscience in Autobiography
Volume 4

Edited by Larry R. Squire
Published by Society for Neuroscience
ISBN: 0-12-660246-8

Jean-Pierre G. Changeux

pp. 116–143

https://doi.org/10.1016/S1874-6055(04)80016-1



Jean-Pierre G. Changeux

BORN:

Domont (95), France
April 6, 1936

EDUCATION:

Ecole Normale Supérieure, Paris (1955)
Paris University (1957)
Institut Pasteur, Ph.D. (1964)

APPOINTMENTS:

Agrégé-Préparateur of Zoology, Ecole Normale Supérieure, Paris (1958)
Sous-Directeur, Collège de France, Paris (1967)
Professor, Collège de France (1975)
Professor, Institut Pasteur (1975)

HONORS AND AWARDS (SELECTED):

Gairdner Foundation Award, Canada (1978)
Richard Lounsbery Prize, National Academy of Sciences, USA (1983)
Wolf Prize, Israel (1983)
Foreign Associate, National Academy of Sciences, USA (1983)
Foreign Member, Royal Academy of Sciences, Stockholm (1985)
Académie des Sciences, Paris (1988)
Bristol-Myers-Squibb Award in Neuroscience (1990)
Carl-Gustaf Bernhard Medal, Swedish Royal Academy of Sciences (1991)
Foreign Member, Academy of Arts and Sciences (1994)
Eli Lilly Award, European College of Neuropsychopharmacology (1999)
Balzan Prize for Cognitive Neuroscience, Berne (2001)
Karl Spencer Lashley Award, American Philosophical Society (2002)

Jean-Pierre Changeux identified and characterized the acetylcholine receptor, demonstrated its allosteric transitions, and described its ion channel. He also helped formalize the concept of synapse selection and competition during development. In addition, building on his broad interests in neuroscience and higher function, he has developed theory, constructed models, and written successfully for the general audience.

Jean-Pierre G. Changeux

Autobiographical accounts are always partial and, therefore, invariably biased. This retrospective examination does not aim at a historical and comparative reconstruction of the development of concepts as general as those of allosteric proteins, the epigenesis of neuronal networks by selection, cognitive learning by reward, or even a faithful description of specific discoveries such as the identification of the acetylcholine receptor. The extraordinary exuberance of scientific research, and the ever-increasing number of scientists throughout the world who contribute either directly or indirectly to the progress of knowledge, and specifically to biological research, are such that, as Carl Popper wrote, "the objectivity of science is not a matter of individuals but a social matter." This objectivity is reached through extensive debates and critics between scientists, through their collaboration as well as through their rivalry. I will therefore limit myself to sketch, in this panoramic synthesis, a personal scientific itinerary that is all but linear, with its successes and failures, its joys and sorrows. Being aware of the collective character of this research and of its multiple dimensions, I would like to start by paying tribute to all those collaborators and colleagues, but also insightful competitors, who have made it possible for this work to exist.

Allosteric Proteins

I owe it to my first mentors, particularly to Jean Bathellier, my Natural Sciences Professor at the Lycée Montaigne, and to Claude Delamare-Debouteville, who opened to me the doors of the Arago Laboratory of Marine Biology at Banyuls-sur-Mer, for encouraging my adolescent's fascination in life sciences and for converting it to a research vocation. By their teaching and by the example they set, they offered me the demonstration that biology is a branch of knowledge by itself, like mathematics, chemistry or physics, and literature, with the added richness of its multidisciplinary dimension.

From the taxonomy of parasitic marine copapods to the study of the fundamental molecular mechanisms of the living cell, the transition is not as abrupt as it seems. My juvenile philosophy, inspired by the work of Jean Brachet and Christian de Duve, whom I met during a training course in Brussels in 1959, was that the great problems of biology, such

as those arising from the evolution of parasites and their unusual embryonic development, were to find their solution at the level of elementary biochemical properties of the egg cell and in the chemistry of its activation by the fertilizing sperm cell thereby eliciting its cleavage and segmentation. My arrival in Jacques Monod's laboratory, at the beginning of 1960, enabled me to put these rather ambitious ideas to the test with the added light shed by structured theoretical reflection and the contribution of rigorous experimentation. Among the several research projects which Jacques Monod and François Jacob proposed for my doctorate thesis, one particularly held my attention. Umbarger and Pardee had shown that in certain bacterial biosynthetic pathways the first enzyme is inhibited, apparently in a 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 fitted directly with the spirit of my first theoretical enthusiasms as a biological student. I therefore selected it as my thesis subject. The experimentation was difficult for a beginner. I felt rather isolated. I tried hard, with L-threonine desaminase, to find a method to dissociate regulatory interaction and catalytic activity *in vitro*. Reagents of thiol groups, thermal treatment, and mutations uncoupled the inhibitory effect caused by isoleucine, while conserving this enzyme's catalytic activity on its substrate. In contrast with the hypothesis of a competitive inhibition, the substrate and the regulatory effector were to bind topographically distinct sites (Changeux, 1961). In the sketch I drew in the paper I presented at the 1961 prestigious Cold Spring Harbor Symposium on Quantitative Biology, the model of "non-overlapping" sites was clearly distinguished from the standard scheme of "overlapping" sites, i.e., mutual inhibition by steric hindrance. The interaction between these two sites was postulated to be indirect, or *allosteric* (a word coined by Monod and Jacob in the Concluding Remarks at the same meeting), and transmitted by a conformational change of the protein molecule (Monod, Changeux, and Jacob, 1963). At this stage of the inquiry, the change was viewed as analogous to the "induced-fit" mechanism suggested long before by Daniel Koshland for the catalytic action of enzymes. In passing, I observed that the sigmoid, cooperative, curve of saturation by the substrate was also uncoupled by the chemical treatment which dissociated regulatory and active sites.

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 desaminase and oxygen binding to hemoglobin. This was the beginning of an exciting epic. My research on the properties of L-threonine desaminase progressed. Later (early 1964), I handed Jacques Monod the first version of my thesis work. Max Perutz's results on hemoglobin's tridimensionnal 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 (Monod, Wyman, and Changeux, 1965) was based on the general principle that the molecular structure of allosteric proteins is organized in a cooperative manner forming "closed microcrystals," or *oligomers*. Furthermore, a mechanism was suggested which links cooperative binding and cooperative structure. It postulated a molecular switch according to which the regulatory protein may exist spontaneously under a small number of discrete conformational states that possess different biological properties. The regulatory signal, under these conditions, *selects* the conformation to which it preferentially binds and shifts the conformational equilibrium, thereby triggering signal transduction. A "Darwinian" selection, rather than a "Lamarckian"-induced-fit-instruction, of conformational states would take place.

In the conclusion of my thesis (1964), I considered the possibility of extending this model to the mechanisms of signal transmission in the nervous system and, more specifically, to the recognition of communication signals at the level of the chemical synapse. The theory was further elaborated together with the solid state physicist Charles Kittel during a first postdoctoral period at the University of California, Berkerley (1966–1967). The possibility was considered that, in membranes, receptors may form highly cooperative assemblies (Changeux et al., 1967). Only recently, Dennis Bray and his colleagues from Cambridge discovered that this mechanism actually takes place in the case of bacterial chemotactic receptors. In any case, these reflexions on membrane receptors were the starting point of investigations that still go on today.

Identification of the Acetylcholine Receptor

At the beginning of the last century, John Newport Langley (1905) postulated the existence of *receptors* engaged in the recognition, and transduction into a physiological response, of drugs or physiological chemical signals, since then called neurotransmitters. The test of the suggested hypothesis that allosteric mechanisms mediate synaptic transmission required the isolation of such a receptor, which since Langley had remained a mysterious entity. I decided to extend my postdoctoral studies in David Nachmansohn's laboratory at Columbia University in New York (1967). During his stay in France at the end of the 1930s, after having fled Nazi Germany, David Nachmansohn discovered the exceptional wealth in biochemical components of the cholinergic synapse of the electric organ from certain fish such as *Torpedo* or *Electrophorus*. He had also set up a preparation of individual cells isolated from the electric organ—or electroplaque—which enables one to investigate electrophysiology, pharmacology, and biochemistry on the same biological system (Nachmansohn, 1959). In his laboratory, I learned to dissect the electroplaque and to record its electrophysiological response

to the neurotransmitter acetylcholine and its derivatives, such as nicotine and curare. Jon Singer, whom I had visited a few months earlier at the University of California, San Diego, had generously offered me a sample of an affinity probe, TDF, which he had previously used with antibodies. This molecule presents a trimethylammonium group, like acetylcholine, as well as a reactive diazonium group. The postulated mechanism was that TDF would bind and irreversibly link itself to the receptor site by a covalent bond. TDF behaved with the electroplaque as expected (Changeux, Podleski, and Wofsy, 1967). This was a significant step in the characterization of the receptor. The receptor was amenable to protein chemistry. This method was soon adopted by Arthur Karlin (1968), who was also a postdoctoral fellow in David Nachmansohn's laboratory. Inspired by earlier work on allosteric enzymes, he had initially observed that the electroplaque response to acetylcholine was sensitive to thiol reagents. On this basis, he improved the method of affinity labeling. However, this specificity soon appeared to be insufficient to allow isolation, from crude electric organ extracts, of the receptor in its active form.

Two singular discoveries allowed this obstacle to be overcome. The first one was the demonstration by Michiki Kasai and myself (1970) that membrane fragments purified from the electric organ have the tendency to reseal and form closed vesicles, or "microsacs." Inspired by the method used by George Cohen and Jacques Monod with bacterial permeases, we were able to measure radioactive Na^+ (or K^+) ion fluxes with the microsacs using a simple filtration method. Even better, the microsacs responded *in vitro* by an increase in ion flux to "nicotinic" cholinergic effectors with a specificity very close to that recorded by electrophysiological methods on the electroplaque and the neuromuscular junction. It thus became possible to study *in vitro* the "chemistry" of the physiological ionic response to acetylcholine. The receptor molecule was present in the purified membranes and I immediately started to try to solubilize it by detergents. 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, almost irreversibly blocks the neuromuscular junction of higher vertebrates at the postsynaptic level. Aware of the Claude Bernard and Louis Pasteur tradition to use toxic compounds as "chemical lancets" to "dissect" physiological mechanisms, I immediately asked him for a sample of this toxin. He accepted, 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 microsacs' ion flux response to nicotinic agonists *in vitro*. It also blocked the *binding* of a nicotinic agonist, decamethonium, to a macromolecule that I had previously solubilized, using a weak detergent, from the microsac preparation. A protein which binds nicotinic agonists and the snake venom toxin in a mutually exclusive

manner could then be identified under a detergent-soluble form that still reversibly binds the neurotransmitter (Changeux, Kasai, and Lee, 1970). In a footnote of the original article, I also mentioned that it could be physically separated from an enzyme long studied in David Nachmansohn's laboratory: acetylcholinesterase. For a while, I thought that acetylcholinesterase might form a supramolecular aggregate with the receptor protein: but this did not happen to be the case. The paper was communicated by Jacques Monod to the Proceedings of the National Academy of Sciences. After its publication, the finding was praised by David Nachmansohn, who for decades had tried to identify the receptor, and by the distinguished Swedish pharmacologist Ulf von Euler. The molecule was shown to be a high molecular weight, hydrophobic protein, strikingly different from acetylcholine esterase and which could be purified in my laboratory (Olsen, Meunier, and Changeux, 1972; Meunier et al., 1974). Examined by electronic microscopy (Cartaud et al., 1973), the receptor molecule resembled some kind of transmembrane "rivet" made up of several subunits organized into a compact bundle and whose synaptic side has the aspect of a rosette with a hydrophilic core. The emotion was immense. For the first time, a neurotransmitter receptor could be "seen."

Molecular Organization of the Acetylcholine Receptor

From then on it became possible to display the intimate organization of the receptor molecule. Was it really an allosteric protein? Was it an oligomer as suggested by the theory? An initial study performed in the laboratory with Ferdinand Hucho (1973) on the purified *Electrophorus electricus* receptor revealed a pentameric organization. I hesitated. The theoretical reflections I had with Jacques Monod stressed the importance of twofold symmetry axes. Indeed, these ideas provided a simple explanation for the evolution of a protein monomer into an oligomer. Nevertheless, these early findings were correct. The teams of Raftery and Karlin, who had no preconceived idea on this particular issue, established the pentameric organization, but also discovered that the structure was more "baroque" than we had expected, raising an additional difficulty for the theory. The receptor molecule resulted from the assembly of four apparently quite different types of subunits organized into a $[2\alpha\beta\gamma\delta]$ pentameric oligomer (see Weill, McNamee, and Karlin, 1974). These subunits had been distinguished by their molecular mass. Nothing was known about their chemistry. Anne Devillers-Thiéry, Dony Strosberg, and myself (1979) then established, using a microsequencing technique, the 20 amino acid sequence of the α-subunit N-terminal domain. It was quickly confirmed by Raftery's team. Today, this result may appear to be rather modest. However, at the time, it had a quite significant impact. A "chemical identity card" of the receptor was henceforth available, the first one ever to be established with a neurotransmitter receptor. It was

quickly confirmed by Raftery's team which, with the help of Leroy Hood's high technology, determined the *four* subunits N-terminal sequence of the Californian *Torpedo* receptor and revealed important sequence identities between the subunits (Raftery et al., 1980). It was a comeback from baroque to classicism. As expected from the Monod, Wyman, and Changeux theory, the receptor protein was indeed an authentic oligomer, but it was *pseudo*-symmetrical, with an unusual fivefold rotation axis perpendicular to the plane of the synaptic membrane.

Basing themselves on these initial sequence data, the groups of Numa, Heinemann, and Barnard, as well as Anne Devillers-Thiéry and Jérôme Giraudat in my laboratory, cloned the complementary DNAs of the different electric organ subunits, and then those of the muscle, and established their complete sequence (Noda et al., 1982; Ballivet et al., 1982; Giraudat et al., 1982; Devillers-Thiéry et al., 1983). The reading of the sequence revealed several functional domains along the subunits sequences: a long hydrophilic N-terminal segment, four hydrophobic segments, and a short cytoplasmic hydrophilic segment, supposedly organized into extracellular (synaptic), transmembrane, and cytoplasmic domains, respectively.

In order to test the hypothesis of an "allosteric" interaction between distinct sites, this time at the *submolecular* level, the respective locations of the acetylcholine binding site and of the ion channel had to be determined. In this second step, the 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 using an affinity labeling reagent of the acetylcholine binding site, which led to the identification of a pair of adjacent cysteins (192–193), located in the N-terminal domain of the α-subunit (1984) (Kao et al., 1984). However, this result did not reveal the site's pharmacological specificity. 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 creates a resonant molecule that can now be photoactivated by energy transfer from the protein. Indeed, our team, in collaboration with that of Hirth and Goeldner from Strasbourg, identified close to eight amino acids labeled by DDF, six of them with an aromatic side chain and all located in the long hydrophilic NH_2 terminal domain. These amino acids are distributed into three main loops (A, B, C), thus forming a sort of electronegative aromatic basket in which acetylcholine quaternary ammonium is capable of lodging itself (Dennis et al., 1988; Galzi et al., 1990). Another important observation shed a new light on the organization of the binding site. It was that the α-toxin, like DDF, labeled the γ- and δ-subunits, in addition to the α-subunit. From this came the idea that the acetylcholine binding site was located at the *interface* between subunits (Oswald and Changeux, 1982). The groups of Cohen, Taylor, and Karlin quickly confirmed this notion by identifying new loops, D, E, and F, located on the "complementary" side of the γ- and δ-subunits. A first validation

of these biochemical results was obtained by directed mutagenesis of the labeled amino acids (see Galzi et al., 1990). However, the most spectacular evidence was recently provided by the Dutch group of Smit and Sixma using crystallographic analysis of a soluble snail protein that binds acetylcholine and happens to be homologous to the receptor's synaptic domain. Most of the amino acids identified by affinity labeling are very precisely found at the acetylcholine binding site level and at the interface between subunits (compare Corringer et al., 2000 and Brecj et al., 2001).

Identification of the Ion Channel

The most difficult task remained: the identification of the ion channel. How would it be possible, using the biochemical methods available, to chemically identify a pore within a protein through which the ions flow? The quest proved to be long and difficult (1974–1999). Relatively old pharmacological observations, made mainly in David Nachmansohn's laboratory and about which I became aware of in 1967 during my stay in his laboratory, inspired the search. Some 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 effect on the receptor binding site. These channel blockers acted, as it were, as a "cork" and offered outstanding tools for channel "labeling." The first step (1974), performed by my student, Michel Weber, and an American postdoctoral fellow, Jonathan Cohen, was to demonstrate, *in vitro*, that the local anesthetics do not directly displace acetylcholine from its site, but bind to a different site (Weber and Changeux, 1974; Cohen, Weber, and Changeux, 1974). The first attempts of reversible binding with a local anesthetic, quinacrine, pointed toward a protein with a molecular mass of 43,000 Da present in the subsynaptic membrane (Sobel, Weber, and Changeux, 1977). However, soon afterward, Jonathan Cohen, who had returned to Harvard University, showed that it was possible to get rid of this protein while conserving the binding of local anesthetics. I then decided to tackle the problem using again the affinity labeling method that was dear to me, but this time with a covalent local anesthetic synthesized in Bernard Roques' laboratory. When we explored the covalent labeling by this photoaffinity probe, Robert Oswald and I (1981) noted that the ultraviolet (UV) irradiation of the control molecule, *without reactive group*, was sufficient to covalently link the molecule to the receptor to the δ-subunit. This unanticipated observation, in fact, enabled us to quickly explore the properties of a large number of potential channel blockers which, because of their aromatic structure, could serve as photolabeling reagents by simple UV irradiation of their complex with the receptor protein. Some among them labeled essentially the δ-subunit, while others labeled several subunits. One of them, chlorpromazine, displayed exceptional properties (1981, 1983). Chlorpromazine labeled the four types of

subunits of the receptor, and this covalent binding was strongly increased by nicotinic agonists and for all subunits at the same time. In addition, the effect of acetylcholine was blocked by d-tubocurarine and α-bungarotoxin. Moreover, my student, Thierry Heidmann, demonstrated that chlorpromazine binds itself to just one high affinity site per [2αβγδ] oligomer (1982, 1983). Furthermore, the kinetics of access to this site increased 100-fold when chlorpromazine was rapidly mixed with acetylcholine, i.e., conditions under which the ion channel opens (Heidmann and Changeux, 1984, 1986). It was then hypothesized that the chlorpromazine binding site is located within the *ion channel*, in the molecule's *pseudo*-symmetry axis (1983, 1984), and 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 MII transmembrane segment (Giraudat et al., 1986). We were in a state of great tension. No one had, until then, suggested that the MII segment could eventually belong to the ion channel. The result was nevertheless made public at the fall meeting of the American Neuroscience Association (1962). We were reassured when, a few months later, Ferdinand Hucho using exactly the same protocol published the same result, but with a different probe. Jérôme Giraudat (1987) and then Frédéric Révah (1989, 1990) (Révah et al., 1990) continued with shrewdness the identification of the chlorpromazine-labeled amino acids on the other subunits. They confirmed, in agreement with Hucho's work, the contribution of a ring of serines but, in addition, 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. The interpretation we made of these results was (1) that the MII segments contribute to the channel walls, (2) that these segments are folded into an α-helix, and (3) that the chlorpromazine binding site is located at a near equatorial position in the channel's pseudo-symmetry axis. The contribution of MII was quickly confirmed and further documented by the teams of Numa and Sakmann (1986, 1988) and Lester and Davidson (1986, 1988) using site-directed mutagenesis and electrophysiological recording techniques after reconstitution in *Xenopus* oocytes, following the method developed by Barnard and Miledi (1982) (cited in Changeux, 1990).

More recent studies, performed in my team by two postdoctoral fellows, Jean-Luc Galzi followed by Pierre-Jean Corringer, in collaboration with Daniel Bertrand from the University of Geneva, have enabled us to progress further. We identified a group of three amino acids that drive, in a critical way, the conversion of the ion channel cationic selectivity into

an anionic one. One of them, which is particularly critical, is located in a loop situated at the cytoplasmic end of the MII segment (Galzi et al., 1992; Corringer et al., 1999). It thus became possible to transform an excitatory acetylcholine receptor into an inhibitory one. The finding has been reproduced by another group with the $5HT_3$ receptor. The converse result—from anionic to cationic—has recently been achieved by other teams, using the same method, with the inhibitory glycine and GABA receptors.

All the data obtained clearly indicate that the receptor sites and the ion channel belong to topographically distinct proteic domains. Their interaction is therefore *allosteric*. Better, Jean-Luc Eiselé, a Swiss researcher working in my laboratory, successfully constructed a functional chimera joining the nicotinic receptor synaptic domain and the $5HT_3$ serotonin receptor transmembrane domain (Eiselé et al., 1993). Therefore, the structural data obtained with the nicotinic receptor could be generalized to other receptors of the "nicotinic family."

Allosteric Transitions of the Acetylcholine Receptor

Additional biochemical results, but of a different nature, brought about additional arguments in favor of the allosteric model. First, at equilibrium, acetylcholine binds in a cooperative manner to its two binding sites that are present in each receptor protein molecule (Weber and Changeux, 1974). Furthermore, application of fast mixing methods derived from Manfred Eigen pioneering studies revealed amazing conformational changes. From Langley's (1905) and Katz and Thesleff's (1957) studies, it was known 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*. Electrical recording methods did not allow a direct measurement of acetylcholine binding to the receptor and therefore rapidly appeared insufficient to investigate the molecular mechanisms of the activation and desensitization transistors. The isolation of a novel generation of microsacs, now extremely rich in receptors (20–40%), from the *Torpedo* electric organ, carried out earlier by Jonathan Cohen, Michel Weber, and myself (1972), opened the door to chemical methods. The extensive kinetic analysis of the fast binding of a fluorescent analog of acetylcholine dansylcholine to these membranes rich in receptors, performed by Thierry Heidmann for his thesis, taught us very much (Heidmann and Changeux, 1979, 1980). It revealed several conformational states of the receptor molecule: the kinetics of interconversion to a state of low affinity corresponded to activation, and several states of high affinity corresponded to desensitization upon rapid mixing with a nicotinic agonist. In contrast to a widespread opinion among pharmacologists, the highest affinity states did not correspond to the active states, but the opposite

was true. Moreover, consistent with the allosteric scheme, a non-negligible fraction (about 20%) of the receptor was spontaneously found in the high affinity, desensitized state. In separate studies of considerable interest, Meyer Jackson (1984) had observed the spontaneous opening of the muscle receptor in the *absence* of acetylcholine. In agreement with the allosteric model, these two series of observations demonstrated that the transition between low and high affinity states of the receptor protein could therefore occur in the *absence* of acetylcholine. However, the situation appeared more complex than for regulatory enzymes. Regarding the receptor, there was not only one, but a cascade of transitions between discrete conformational states. I took the opportunity to generalize the conclusion to possible mechanisms of synaptic plasticity. In a short theoretical model, I suggested together with Thierry Heidmann (1982) that the characteristic property by which neurotransmitter receptors undergo multiple allosteric transitions with different time scales could be involved in the regulation of synaptic strength, particularly in elementary learning mechanisms. The idea deserves, in my opinion, some consideration as an alternative to the NMDA receptor-Mg^{2+} plug device. Indeed, it could apply to all the other receptors, including the non-NMDA glutamate receptors that display desensitization.

Gain of Function Mutations and Receptor Diseases

These thoughts opened the door, as will be described later, to the idea of a possible contribution of allosteric receptors to higher brain functions. An unanticipated discovery brought an additional dimension: that 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 the nicotinic brain receptors, had identified in the chick a new subunit type, which they named α7 (Couturier et al., 1990). Like the other neuronal receptor subunits, it presented important sequence identities with the muscle receptor, but appeared to be more archaic. It possessed the remarkable ability to associate with itself into a homomeric functional receptor, after expression in *Xenopus* oocytes. At last, the demonstration of a nicotinic receptor with perfect symmetry could be established, as expected from the original allosteric model. I immediately recognized that this system was the most appropriate to investigate the functional role of the amino acids homologous to those chemically identified by affinity labeling in *Torpedo*. Marc Ballivet agreed to give us an α7 cDNA. I asked my student, Frédéric Révah, to specifically mutate the chlorpromazine-labeled amino acids in α7 (Revah et al., 1991). The first recordings performed by Daniel Bertrand surprised us. Indeed, the mutation of leucine 247 into threonine did not cause an expected loss of channel function, but, on the contrary, resulted in a "gain of function": a dramatic decrease in the desensitization rate and, in addition, a near to 100-fold increase in apparent

affinity. How strange! While discussing these results at a laboratory meeting, an interpretation suddenly came to my mind. The simplest explanation for these effects could originate from the allosteric model, assuming, for instance, that the high affinity desensitized state becomes permeable to ions. If this were the case, any molecule stabilizing the desensitized state should potentiate the response. I then recalled pioneering studies performed in my laboratory by Hans Grünhagen, one of Manfred Eigen's former students, and the discovery that antagonists, such as curare, could stabilize the desensitized state (Grünhagen and Changeux, 1976). If this happened to be the case, we would predict that α7 receptor antagonists, such as dihydro-β-erythroidine, could act like agonists. I called Daniel Bertrand to share this idea with him. He called me back a few days later. Effectively, dihydro-β-erythroidine behaved as an agonist on the L247T receptor (Bertrand et al., 1992). According to me, these results provided additional evidence in favor of a mechanism of allosteric transition between "rigid" states, which would preexist before the interaction with the ligand.

We were even more happily surprised when, in a totally independent way, Andrew Engel, Steven Sine, and their colleagues at the Mayo Clinic in the United States (see Engel and Ohno, 2002) subsequently reported that in some (not all) of the 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. Among the 13 mutations associated with this phenotype, 7 were located in MII, 1 of which was precisely at the homologous position of leucine 247. Stuart Edelstein, Professor at the University of Geneva, whom I had met in Berkeley in 1966, came to visit me at the Pasteur Institute in order to reexamine the application and the generalization of the allosteric model to the known neurotransmitter receptors and ion channels (Edelstein et al., 1996). The quantitative examination of the properties of mutant receptors from myasthenic patients revealed that their properties were exquisitely fitted by the allosteric model (1996, 1997) (Edelstein et al., 1997). The phenomenon can be extended to other receptors. A whole class of "receptor diseases" which include G-protein-linked receptors and tyrosine kinase receptors may be directly caused by the perturbation of the allosteric properties of these receptors (Changeux and Edelstein, 1998).

Epigenesis by Selective Synaptic Stabilization

Parallel to my molecular biology studies on regulatory enzymes and, later, on the acetylcholine receptor, I could not avoid returning to my youth "dreams" on the chemistry of embryonic development. We were in 1970. Jacques Monod had finished writing *Chance and Necessity*. I read the book with great interest, but also with the critical distance of a student who had become somewhat "parricidal," as Jacques Monod wrote in the affectionate

dedication of the copy he gave me. Although I greatly shared the philosophy, I found his position on the development of the central nervous system too much based on innate influences. Well informed admirer of Wiesel and Hubel's work on the effects of experience on the postnatal development of the visual cortex (1965), I did not share their views on the "functional validation" by experience of preformed innate patterns of nerve connections. At a meeting organized by Edgar Morin on "l'Evénement," I suggested instead that exuberant and variable distribution of connections would become established through some kind of trial-and-error process and that at "critical" or sensitive periods a *synaptic* selection would occur according to a Darwinian epigenetic mode under the control of network activity (Changeux, 1972). The idea that regressive processes co-occur with mechanisms of synaptic competition during development had already been mentioned on several occasions since Ramón y Cajal (1899). However, this concept had neither been mathematically formalized nor generalized. Philippe Courrège, Antoine Danchin, and myself tried hard to accomplish this (Changeux, Courrège, and Danchin, 1973; Changeux and Danchin, 1976), and this attempt yielded two major consequences. First, the demonstration that a particular spatial and temporal distribution of electrical and chemical activity in a developing network is liable to be inscribed under the form of a particular and stable topology of connections within what I called a "genetic envelope." Second, the proposition, presented as a theorem of "variability," that the selection of networks having different connective topologies can lead to the same input–output behavioral relationship.

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 debate. Even if the Darwinian metaphor raises discussions, its application to higher levels of organization, known as cognitive, enriched debates with Gerald Edelman, Terrence Sejnowski, Jeff Lichtman, and Dale Purves. Among its benefits, it gave rise to new models, both experimental and theoretical: "top-down" as well as "bottom-up."

An example of the bottom-up model was the junction between the motor nerve and the skeletal muscle, the simplest experimental model of chemical synapse whose anatomy (Couteaux, 1978), physiology (Katz, 1966), and biochemistry (Nachmansohn, 1959), particularly that of its principal component, the acetylcholine receptor (Changeux et al., 1970), were now known.

At the presynaptic level, Redfern (1970) had shown that, during the development of the motor endplate, a multiple innervation with three to five nerve endings occurs at birth and disappears later, since only one motor axon per muscle fiber remains in the adult. During his postdoctoral studies in my laboratory, Pierre Benoit (Benoit and Changeux 1975, 1978) demonstrated, for the first time, that in the newborn rat the state of activity of

the junction controls the elimination of supernumerary terminals. Following my suggestion, Francis Crépel and Jean Mariani (1976, 1981) extended this observation to the development of the innervation of cerebellar Purkinje cells by climbing fibers. Later, other groups produced important experimental data in favor of the selectionist model (Lichtman, Constantine-Paton, Stryker, Shatz, etc.). The eventual contribution of instructive Lamarckian processes to the postnatal development of brain networks, yet, is still debated (Sejnowski, Purves).

The studies on the cerebellar mutants led to a casual observation which unexpectedly opened a new area of research. Looking at the protein compositions of the cerebellum of mutant mice deprived of Purkinje cells (Mallet et al., 1974), a freshly arrived postdoctoral fellow, Jacques Mallet, found that a high molecular weight band (called P400) was missing. Katsuhiko Mikoshiba, a very dynamic postdoctoral fellow, confirmed the observation (Mikoshiba, Huchet, and Changeux, 1979) and back in Japan built from it the splendid story of the IP3 receptor (Nikoshiba, 2003).

Molecular Morphogenesis of the Synapse

The model of epigenesis by selection aroused, in parallel, new investigations on the differentiation of the *postsynaptic* domain using the significant contribution of knowledge acquired about the molecular biology of the acetylcholine receptor. In particular, α-bungarotoxin allowed (in an electronic 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 (around 15,000/μm^2) and persists several weeks after denervation (Bourgeois et al., 1972, 1978). The α-toxin was also an exceptional tool in the hands of John Merlie [an American postdoctoral fellow who I converted from bacteriology to neurobiology and collaborated with in François Gros' laboratory (Merlie et al., 1975; Merlie, Changeux, and Gros, 1978)] and also in the hands of Heinrich Betz (my second German postdoctoral fellow) to study the biosynthesis of the muscle receptor. Its repression by electrical activity was demonstrated during muscle development (Betz, Bourgeois, and Changeux, 1977; Bourgeois et al., 1978).

A new conceptual stage was reached with the analysis, using molecular genetics methods, 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 (1978) had noticed that the muscle nuclei lying directly under the motor nerve terminal presented a very unusual anatomy and, therefore, named them "fundamental nuclei." John Merlie and Josh Sanes (1984) had also noticed that innervated muscle regions were richer in messenger RNAs coding for the receptor subunits than the non-junctional 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) (Fontaine et al., 1988; Fontaine and Changeux, 1989), that these messenger RNAs are strictly located at the level of the fundamental nuclei. There is a "compartmentalization" of the expression of the receptor genes at the level of the subneural domain. Several groups (Goldman, Brenner, Sakmann, Burden) confirmed this observation. It enabled the analysis, conducted in my laboratory throughout the years by several students and postdoctoral fellows (André Klarsfeld, 1987; Jacques Piette, 1989, 1990; Jean-Louis Bessereau, 1994; Satoshi Koike, 1996; and Laurent Schaeffer, 1998), of the genetic mechanisms (DNA elements and transcription factors) that regulate this elementary morphogenesis. For instance, we discovered that distinct genetic determinants and signaling systems control the targeting of transcription under the synapse (*N Box*) via trophic factors of neural origin and the repression by electrical activity outside the synapse (*E Box*) (Schaeffer et al., 2001). It lead to the identification of an Ets transcription factor as a crucial element for the normal formation of the neuromuscular junction (De Kerchove d'Exaerde et al., 2002).

The posttranscription stages, studied by Jean Cartaud and colleagues in collaboration with my group, i.e., the transit through a specialized Golgi apparatus (1989), a particular secretory pathway (1990, 1995), and the assembly, by the 43K-Rapsyn protein (discovered in my laboratory by André Sobel in 1977), into sub-synaptic aggregates, confirmed and extended the model of a progressive compartmentalization of gene expression in the course of the formation of the neuro-muscular synapse.

Michel Kerszberg and I (1993) then had sufficient data in hand to describe this process as a cybernetic mathematical model that accounts for the formation of a sharp boundary of gene transcription, during the development of the motor endplate. Further development of the model accounts for the positioning of this boundary in a morphogenesis gradient during embryonic development (Kerszberg and Changeux, 1994, 1998). The mechanism was soon extended to the basic issue of the formation and parcellization of the neural plate in the course of embryonic development, which potentially plays a crucial role in the phylogenesis of vertebrate brain (Kerszberg and Changeux, 1998).

Nicotinic Receptors in the Brain

Naturally, the strategy of understanding neural development at the level of networks of transcription factors linking dispersed populations of promoter elements was to be applied to the expression of neuronal nicotinic receptor genes in the brain. Clarke, Patrick, Heinemann, and others had described the distinct topological distribution of the various types of neuronal nicotinic receptors and of the messenger RNAs of their subunits in

the brain. Michele Zoli, a postdoctoral fellow from Italy, carefully examined during development the expression of the α3, α4, β2, β4 genes which starts very early at day 10 of embryonic development and can be synchronous in certain regions (spinal cord), but not in others (cerebral cortex) (Zoli et al., 1995). This required a particularly sophisticated transcriptional regulation. Indeed, my student Alain Bessis (Bessis et al., 1993, 1997) showed, in the case of the genes for the α2 and β2 subunits, that the regulation of their transcription is submitted to a complex interplay between activatory regulatory sequences and inhibitory ones. Moreover, the fine analysis of nicotinic receptor distribution at the neuronal level using electrophysiological methods, performed by my collaborators Christophe Mulle (Mulle and Changeux, 1990; Mulle et al., 1991) and Clément Léna (Léna, Changeux, and Mulle, 1993; Léna and Changeux, 1997), indicated that the receptor protein is not only distributed on the neuronal soma and dendrites, but also on the axonal terminals and on the segments located close to the terminal referred to as preterminal (Léna, Changeux, and Mulle, 1993). A critical question arose: what are the functions of the various brain nicotinic receptor forms associated with such a complex organization? Further theoretical reflection was needed.

Neuronal Man and Cognitive Learning by Reward

In 1983 I published *Neuronal Man: The Biology of Mind.* This book covered the contents of the first seven years of lectures I gave at the Collège de France and was enriched by the laboratory's current research. I ventured to collect and critically synthesize the data which had been gathered thanks to extraordinary progress in neuroscience since the 1970s, from the molecular and cellular levels to cognitive functions, even consciousness. In it, I substantiated and documented the thesis of epigenesis by synaptic selection. In the chapter on "Mental Objects," I extended it to higher brain functions. I developed Hebb's old proposal according to which the representations formed by our brain can be identified with activity states of "cooperative" neuron assemblies. I integrated it with the selectionist model, proposing that acquisition of knowledge, in other words the neuronal inscription of meaning, is carried out in at least two steps: the genesis of multiple and transitory "prerepresentations" followed by the selection of the "adequate" representation(s) of the outside world. The first selection mechanism that was retained was that of "resonance" between prerepresentations of internal origin and the percept evoked by an interaction with external reality (1983).

While realizing the need for deeper analysis, particularly at the cognitive level, I decided to discuss the issue in the mid-1980s with my close friend Jacques Mehler, whose experience with psycholinguistics could be

a great source of enrichment. He put me in contact with one of his students, Stanislas Dehaene, who had been trained in mathematics at the Ecole Normale Supérieure but was doing experimental psychology under his supervision. It was the starting point of an exceptionally fruitful collaboration which still continues actively today. Stanislas and I agreed about two major issues from the start: (1) a theoretical model had meaning only if it concerned a defined behavioral task, accessible to experimentation; and (2) the formal model should be based on plausible neuronal data. It should be as "neuro-realistic" as possible. 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 (Dehaene, Changeux, and Nadal, 1987).

We then decided to extend the modeling to more elaborate cognitive functions and, in particular, to tasks with which I became acquainted during my teaching at the Collège de France. We selected the well-known delayed-response tasks which, in mammals, mobilize the frontal cortex. New modes of selection had to be found at the cognitive level. The involvement of "reward" processes, suggested by Thorndike, Pavlov, and Skinner, in an "empirical" context seemed plausible and adaptable to the selectionist scheme. In addition, this idea enriched the modeling work with a new biochemical dimension. Neuronal systems specialized in reward and punishment had been identified for years. They engaged specific neurotransmitters such as dopamine and serotonin, as well as acetylcholine. Hence, we developed and formalized the idea (Dehaene and Changeux, 1989, 1991) that the prerepresentations produced by a neuronal "generator of diversity" could be selected by the release of a 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 mechanism of modulation of synaptic strength by the reward signal. This still hypothetical mechanism was derived from the extension of the allosteric scheme that I proposed with Thierry Heidmann in 1982 of a coincidence reading of two synaptic signals—including a neurotransmitter of reward—by a common allosteric state of the synaptic receptors. In computer experiments, the virtual organisms, constructed on this basis, passed the task. Others were designed for the Wisconsin Card Sorting test (1991) or even the Tower of London task (1997). The models accounted for the expected cognitive behaviors, but also offered many new experimental predictions.

The *in vivo* techniques of gene invalidation in mice provided original experimental avenues to put to the test our hypotheses and computer models that covered intricate organization levels from molecules to cognitive functions. This approach in fact enabled us to approach the role of the various neuronal nicotinic receptor subunits. I entrusted to Marina Picciotto, an American postdoctoral fellow, the task of constructing a mouse invalidated

for the β2 subunit, the most largely distributed subunit in the brain. After several difficult years, with the help from several colleagues at the Pasteur Institute and with great courage, she succeeded (Picciotto et al., 1995). The mutant mouse displayed quite peculiar behavioral traits. It no longer responded to nicotine in a passive avoidance learning task, and also showed alterations in reward processes. For instance, nicotine self-administration as well as the effect of nicotine on dopamine release were abolished (Picciotto et al., 1998). The anti-nociceptive effect of nicotine was also lost in the β2 mutant mouse, as well as in the α4 mutant mouse (also constructed by Lisa Marubio, another American postdoctoral fellow). These mice possessed somewhat altered punishment mechanisms. These results are still far from giving a fair evaluation of the suggested models. Yet, they open fruitful experimental perspectives regarding nicotine addiction, analgesia, and cognitive functions.

Conscious Space and Nicotinic Receptors

In *Neuronal Man*, I tackled the issue of consciousness and the neuronal bases of "becoming conscious" and stated that the relevant explanation had to be found at the level of a system of neuronal regulations functioning as a global entity. In my 1992 course at the Collège de France, I suggested that the formal neuronal network that Stanislas Dehaene and myself had proposed for the Wisconsin Card Sorting task could serve as a starting point for the development of a more general model that would include a "conscious workspace." Two events prompted us to tackle this modeling in a direct way. On the one hand, Stanislas Dehaene had created a very active independent research group dedicated to brain imaging and started to apply this technique to investigate conscious versus nonconscious tasks. On the other hand, Antonio Coutiño gave us the opportunity to present our ideas in Portugal to a group of experts brought together by the Gulbenkian Foundation in the monastery of Arrabida in the summer of 1998. Our position differed from Francis Crick's 40-Hz reductionism, from Gerald Edelman's complexity dialectic, and from Rodolfo Llinás' thalamocortical oscillations. Rather, our intention was to imagine a neuronal architecture that would explain altogether the global and unitary character of the conscious workspace, as suggested by the psychologist Baars (1989) *and* the diversity of the underlying processes. It was elaborated as a computer model. Dehaene, Kerszberg, and myself (1998) proposed that neurons with long axons connecting distinct cortical areas, even different hemispheres, play an essential role in the genesis of the conscious space. In effortful tasks, such as the Stroop test, "global" representations would differentially mobilize these neurons together with components from specialized processes through mechanisms of evaluation from the outside world, but also through mechanisms of *self-evaluation* toward the subjective *inner world*. The proposed computer model

is able to successfully simulate the Stroop task. The model also accounts for the top-down control of these global representations upon the activity states of the underlying processes by a simple neuronal mechanism. A tentative answer was brought, in neuronal terms, to the paradox raised by Sperry of the mysterious top-down control of consciousness over lower neuronal processes.

Von Economo's (1929) studies on the microarchitecture of the cerebral cortex underlined the abundance of pyramidal neurons with long axons in layers II and III of the cerebral cortex. Interestingly, these layers are specially dense in the so-called association areas which include the prefrontal cortex. Moreover, brain imaging studies, particularly those performed by Stanislas Dehaene and his group, revealed a strong activation of the prefrontal areas during the accomplishment of conscious tasks requiring an effort. Finally, everyone knows about nicotine's effects on wakefulness and on attention.

Was it reasonable to link these theoretical thoughts and the experimental studies carried out with the neuronal receptor? A first link was established by a discovery made by the Australian neurologist Bercovic and the German molecular biologist Steinlein (Steinlein et al., 1995). Bercovic had recognized that several members of the same Australian family suffered from a rare form of autosomal dominant nocturnal frontal lobe epilepsy, which causes loss of consciousness and convulsions. This was the first genetic epilepsy to be identified at the amino acid level; moreover, it resulted from a mutation of the gene coding for the $\alpha 4$ subunit of the acetylcholine nicotinic receptor. My surprise and delight was 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 that we had initially labeled with chlorpromazine in the *Torpedo* receptor! Spontaneous mutations revealed, independently of any preconceived idea, the *same amino acid* as the one we had labeled in a deliberate way to identify the ion channel. Can a more "objective" validation of these results be conceived?

The subject of the neural bases of consciousness and of its chemistry is henceforth opened to scientific research. The nicotinic receptor may again play a role. Is it a new start for the chemistry of consciousness and cognitive functions? It will require, in order to progress, a multidisciplinary approach that unites life sciences and human sciences at multiple levels of organization, together with human sciences. It is our duty and that of the younger generations to make it work. We are far from the end ....

Bibliographic references of the team can be found on the laboratory's Web site: http://www.pasteur.fr/recherche/unites/neubiomol/bibliography.html. Additional references on the history of the acetylcholine receptors can be found in Changeux references (1981, 1990). This paper is inspired by the document delivered for the Balzan prize ceremony (2000).

Selected Bibliography

Baars BJ. *A cognitive theory of consciousness*. Cambridge, UK: Cambridge University Press, 1989.

Ballivet M, et al. Molecular cloning of cDNA coding for the gamma-Subunit of Torpedo acetylcholine receptor. *Proc Natl Acad Sci USA* 1982;79:4466–4470.

Barnard EA, Miledi R, Sumikawa K. Translation of exogenous messenger RNA coding for nicotinic acetylcholine receptors produces functional receptors in *Xenopus oocytes*. *Proc R Soc (Lond)* 1982;215:241–246.

Benoit P, Changeux JP. Consequences of tenotomy on the evolution of multiinnervation in developing rat soleus muscle. *Brain Res* 1975;99:354–358.

Benoit P, Changeux JP. Consequences of blocking nerve activity on the evolution of multi-innervation at the regenerating neuromuscular junction of the rat. *Brain Res* 1978;149:89–96.

Bertrand D, Devillers-Thiéry A, Revah F, Galzi JL, Hussy N, Mulle C, Bertrand S, Ballivet M, Changeux JP. Unconventional pharmacology of a neuronal nicotinic receptor mutated in the channel domain. *Proc Natl Acad Sci USA* 1992; 89:1261–1265.

Bessereau JL, Stratford-Perricaudet L, Piette J, Le Poupon C, Changeux JP. In vivo and in vitro analysis of electrical activity-dependent expression of muscle acetylcholine receptor genes using adenovirus. *Proc Natl Acad Sci USA* 1994;91:1304–1308.

Bessis A, et al. Negative regulatory elements upstream of a novel exon of the neuronal nicotinic acetylcholine receptor alpha2 subunit gene. *Nuclei Acid Res* 1993;21:2185–2192.

Bessis A, et al. The neuron-restrictive silencer element (NRSE): A dual enhancer/silencer crucial for patterned expression of a nicotinic receptor gene in the brain. *Proc Natl Acad Sci USA* 1997;94:5906–5911.

Betz H, Bourgeois JP, Changeux JP. Evidence for degradation of the acetylcholine (nicotinic) receptor in skeletal muscle during the development of the chick embryo. *FEBS Lett* 1977;77:219–224.

Bourgeois JP, et al. Localization of the cholinergic receptor protein in Electrophorus electroplax by high resolution autoradiography. *FEBS Lett* 1972;25:127–133.

Bourgeois JP, et al. Quantitative studies on the localization of the cholinergic receptor protein in the normal and denervated electroplaque from Electrophorus electricus. *J Cell Biol* 1978;79:200–216.

Brejc K, Van Dijk WJ, Klaassen RV, Schuurmans M, Van Der Oost J, Smit AB, Sixman TK. Crystal structure of an ACh-binding protein reveals the ligand binding domain of nicotinic receptors. *Nature* 2001;411:269–276.

Brejc K, Van Dijk WJ, Klaassen R, Schuurmans M, van der Oost J, Smit AB, and Sixman TK. Crystal structure of AchBP reveals the ligand-binding domain of nicotinic receptors. *Nature* 2001;411:269–276.

Brodmann K. *Vergleichende Lokalisationslehre des Groshirnrinde in ihren Preinzipien dargestellt auf Grund des Zellenbaues*. Leipzig: Barth, 1909.

Cartaud J, et al. Presence of a lattice structure in membrane fragments rich in nicotinic receptor protein from the electric organ of Torpedo marmorata. *FEBS Lett* 1973;33:109–113.

Changeux JP. The feedback control mechanism of biosynthetic L-threonine deaminase by L-isoleucine. *Cold Spring Harbor Symp Quant Biol* 1961;26:313–318.

Changeux JP. Le cerveau et l'événement. *Communications* 1972;18:37–47.

Changeux JP. The acetylcholine receptor: An allosteric membrane protein. *The Harvey Lectures* 1981;75:85–254.

Changeux JP. *L'Homme neuronal*. Paris: Fayard, 1983;419.

Changeux JP. Functional architecture and dynamics of the nicotinic acetylcholine receptor: An allosteric ligand-gated ion channel. Fidia Research Foundation Neuroscience Award Lectures, Volume 4, 1990;21–168.

Changeux JP, Courrège P, Danchin A. A theory of the epigenesis of neural networks by selective stabilization of synapses. *Proc Natl Acad Sci USA* 1973;70:2974–2978.

Changeux JP, Danchin A. Selective stabilization of developing synapses as a mechanism for the specificication of neuronal networks. *Nature* 1976;264:705–712.

Changeux JP, Kasai M, Lee CY. The use of snake venom toxin to characterize the cholinergic receptor protein. *Proc Natl Acad Sci USA* 1970;67:1241–1247.

Changeux JP, Dehaene S. Hierarchical neuronal modeling of cognitive functions: From synaptic transmission to the Tower of London. *CR Adad Sci Paris* 1998;321:241–247.

Changeux JP, Edelstein S. Allosteric receptor after 30 years. *Neuron* 1998;21: 959–980.

Changeux JP, Podleski T, Wofsy L. Affinity labeling of the acetylcholine receptor. *Proc Natl Acad Sci USA* 1967;58:2063–2070.

Changeux JP, Thiéry J, Tung Y and Kittel C. On the cooperativity of biological membranes. *Proc. Natl. Acad. Sci. Wash.* 1967;57:335–341.

Cohen JB, Weber M, Changeux JP. Effects of local anesthetics and calcium on the interaction of cholinergic ligands with the nicotinic receptor protein from Torpedo marmorata. *Mol Pharmacol* 1974;10:904–932.

Corringer PJ, Bertrand S, Galzi JL, Devillers-Thiéry A, Changeux JP, Bertrand D. Mutational analysis of the charge selectivity filter of the alpha 7 nicotinic acetylcholine receptor. *Neuron* 1999;22:831–843.

Corringer PJ, Le Novère N, Changeux JP. Nicotinic receptors at the amino acid level. *Annu Rev Pharmacol* 2000;40:431–458.

Couteaux R. *Recherches morphologiques et cytochimiques sur l'organisation des tissus excitables*. Paris: Robin et Marenge, 1978;225.

Couturier S, Bertrand D, Matter JM, Hernandez MC, Bertrand S, Millar N, Valera S, Barkas T, Ballivert M. A neuronal nicotinic acetylcholine receptor subunit (alpha7). Is developmentally regulated and forms a homo-oligomeric channel blocked by alpha-BTX. *Neuron* 1990;5:847–856.

Crépel F, Mariani J, Delhaye-Bouchaud N. Evidence for a multiple innervation of Purkinje cells by climbing fibers in immature rat cerebellum. *J Neurobiol* 1976;7:567–578.

De Kerchove d'Exaerde A. Cartaud J, Ravel-Chapuis A, Seroz T, Pasteau F, Angus LM, Jasmin B, Changeux JP and Schaeffer L. Expression of mutant Ets protein at the neuromuscular synapse causes alterations in morphology and gene expression. *EMBO Reports* 2002;3:2201–2207.

Dehaene S, Changeux JP. A simple model of prefrontal cortex function in delayed-response tasks. *J Cognitive Neurosci* 1989;1:244–261.

Dehaene S, Changeux JP. The Wisconsin card sorting test: Theoretical analysis and simulation of a reasoning task in a model neuronal network. *Cerebral Cortex* 1991;1:62–79.

Dehaene S, Changeux JP. A hierarchical neuronal network for planning behavior. *Proc Natl Acad Sci USA* 1997;94:13293–13298.

Dehaene S, Changeux JP, Nadal JP. Neural networks that learn temporal sequences by selection. *Proc Natl Acad Sci USA* 1987;84:2727–2731.

Dehaene S, Kerszberg M, Changeux JP. A neuronal model of a global workspace in effortful cognitive tasks. *Proc Natl Acad Sci USA* 1998;95:14529–14534.

Dennis M, Giraudat J, Kotzyba-Hibert F, Goeldner M, Hirth C, Chang JY, Lazure C, Chrétien M, Changeux JP. Amino acids of the Torpedo marmorata acetylcholine receptor subunit labeled by a photoaffinity ligand for the acetylcholine binding site. *Biochemistry* 1988;27:2346–2357.

Devillers-Thiéry A, et al. The amino-terminal sequence of the 40K subunit of the acetylcholine receptor protein from Torpedo marmorata. *FEBS Lett* 1979;104:99–105.

Devillers-Thiéry A, et al. 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* 1983;80:2067–2071.

Edelman G. *Neural Darwinism: The theory of neuronal group selection*. New York: Basic Books, 1987.

Edelstein S, Changeux JP. Allosteric proteins after 30 years: The binding and state functions of the neuronal alpha 7 nicotinic acetylcholine receptor. *Experientia* 1996;52:1083–1090.

Edelstein S, Schaad O, Changeux JP. Single binding versus single channel recordings: A new approach to study ionotropic receptors. *Biochemistry* 1997;36:13755–13760.

Edelstein S, Schaad O, Henry E, Bertrand D, Changeux, JP. A kinetic mechanism for nicotinic acetylcholine receptors based on multiple allosteric transitions. *Biol Cybern* 1996;75:361–379.

Eiselé JL, Bertrand S, Galzi JL, Devillers-Thiéry A, Changeux, JP, Bertrand D. Chimaeric nicotinic-serotonergic receptor combines distinct ligand binding and channel specificities. *Nature* 1993;366:479–483.

Engel AG, Ohno K. Congenital myasthenic syndromes. *Adv Neurol* 2002;88:203–215.

Fontaine B, Changeux JP. Localization of nicotinic acetylcholine receptor alpha-subunit transcripts during myogenesis and motor endplate development in the chick. *J Cell Biol* 1989;108:1025–1037.

Fontaine B, et al. 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* 1988;7:603–609.

Galzi JL, Revah F, Black D, Goeldner M, Hirth C, Changeux JP. Identification of a novel amino acid alpha-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* 1990;265:10430–10437.

Giraudat J, Dennis M, Heidmann T, Chang JY, Changeux JP. Structure of the high affinity site for noncompetitive blockers of the acetylcholine receptor: Serine-262 of the delta subunit is labeled by [^{3}H]-chlorpromazine. *Proc Natl Acad Sci USA* 1986;83:2719–2723.

Giraudat J, Devillers-Thiéry A, Auffray C, Rougeon F, Changeux JP. Identification of a cDNA clone coding for the acetylcholine binding subunit of Torpedo marmorata acetylcholine receptor. *EMBO J* 1982;1:713–717.

Galzi JL, Devillers-Thiery A, Hussy N, Bertrand S, Changeux JP, Bertrand D. Mutations in the ion channel domain of a neuronal nicotinic receptor convert ion selectivity from cationic to anionic. *Nature* 1992;359:500–505.

Galzi JL, et al. Functional significance of aromatic amino acids from three peptide loops of the alpha 7 neuronal nicotinic receptor site investigated by site-directed mutagenesis. *FEBS Lett* 1991;294:198–202.

Goldman D, Brenner HR, Heinemann S. Acetylcholine receptor alpha-, beta-, gamma-, and delta-subunit mRNA levels are regulated by muscle activity. *Neuron* 1988;1:329–335.

Goldman D, et al. Muscle denervation increases the levels of two mRNAs coding for the acetylcholine receptor alpha-subunit. *J Neurosci* 1985;5:2553–2558.

Grünhagen HH, Changeux JP. Studies on the electrogenic action of acetylcholine with Torpedo marmorata electric organ. Quinacrine: A fluorescent probe for the conformational transitions of the cholinergic receptor protein in its membrane bound state. *J Mol Biol* 1976;106:497–516.

Heidmann O, et al. Chromosomal localization of muscle nicotinic acetylcholine receptor genes in the mouse. *Science* 1986;234:866–868.

Heidmann T, Bernhardt J, Neumann E, Changeux JP. Rapid kinetics of agonist binding and permeability response analyzed in parallel on acetylcholine receptor-rich membranes from Torpedo marmorata. *Biochemistry* 1983;22:5452–5459.

Heidmann T, Changeux JP. Fast kinetic studies on the interaction of a fluorescent agonist with the membrane-bound acetylcholine receptor from T. marmorata. *Eur J Biochem* 1979a;94:255–279.

Heidmann T, Changeux JP. Fast kinetic studies on the allosteric interactions between acetylcholine receptor and local anesthetic binding sites. *Eur J Biochem* 1979b;94:281–296.

Heidman T, Changeux JP. 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 Commun* 1980;97:889–896.

Heidmann T, Changeux JP. Un modèle moléculaire de régulation d'efficacité d'un synapse chimique au niveau postsynaptique. *CR Acad Sci Paris* 1982;3,295: 665–670.

Heidmann T, Changeux JP. 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* 1984;81:1897–1901.

Heidmann T, Changeux JP. Characterization of the transient agonist-triggered state of the acetylcholine receptor rapidly labeled by the noncompetitive blocker [^{3}H]chlorpromazine: Additional evidence for the open channel conformation. *Biochemistry* 1986;25:6109–6113.

Heidmann T, Oswald RE, Changeux JP. Le site de liaison de haute affinité de la chlorpromazine n'est présent qu'à un seul exemplaire par molécule de réceptor cholinergique et est commun aux quatre chaînes polypeptidiques. *CR Acad Sci Paris* 1982;295:345–349.

Imoto K, Busch C, Sackmann B, Mishina M, Konno, T, Nakai J, Bujo H, Mori Y, Fukuda K, Numa, S. Rings of negatively charged amino acids determine the acetylcholine receptor channel conductance. *Nature* 1988;335:645–648.

Jackson MB. Spontaneous openings of the acetylcholine receptor channel. *Proc Natl Acad Sci USA* 1984;81:3901–3904.

Jasmin BJ, Cartaud J, Bornens M, Changeux JP. Golgi apparatus in chick skeletal muscle: Changes in its distribution during endplate development and after denervation. *Proc Natl Acad Sci USA* 1989;86:7218–7222.

Karlin A, Cowburn DA. The affinity labelling of partially purified acetylcholine receptor from electric tissue of Electrophorus. *Proc Natl Acad Sci USA* 1973;70:3636–3640.

Karlin A, Winnick M. Reduction and specific alkylation of the receptor for acetylcholine. *Proc Natl Acad Sci USA* 1968;60:668–674.

Kao P, Dwork A, Kaldany R, Silver M, Wideman J, Stein S, Karlin A. Identification of the alpha-subunit half-cystine specifically labeled by an affinity reagent for the acetylcholine receptor binding site. *J Biol Chem* 1984;259:11662–11665.

Kasai M, Changeux JP. Demonstration de l'excitation par des agonistes cholinergiques à partir de fractions de menbranes purifiées *in vitro*. *CR Acad Sci Paris* 1970;270D:1400–1403.

Katz B. *Nerve muscle and synapse*. New York: McGraw Hill, 1966.

Katz B, Thesleff S. A study of the desensitization produced by acetylcholine at the motor end-plate. *J Physiol* 1957;138:63–80.

Kerszberg M, Changeux JP. A model for motor endplate morphogenesis: Diffusible morphogens, transmembrane signalling and compartmentalized gene expression. *Neural Computation* 1993;5:341–358.

Kerszberg M, Changeux JP. A model for reading morphogenetic gradients: Autocatalysis and competition at the gene level. *Proc Natl Acad Sci USA* 1994;91:5823–5827.

Kerszberg M, Changeux JP. A simple molecular model of neurulation. *BioEssay* 1998;20:758–770.

Klarsfeld A, et al. A 5′ flanking region of the chicken acetylcholine receptor alpha-subunit gene confers tissue-specificity and developmental control of expression in transfected cells. *Mol Cell Biol* 1985;7:951–955.

Koike S, Schaeffer L, Changeux JP. Identification of a DNA element determining synaptic expression of the mouse acetylcholine receptor delta-subunit gene. *Proc Natl Acad Sci USA* 1995;92:10624–10628.

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

Léna C, Changeux JP, Mulle C. Evidence for "Preterminal" nicotinic receptors on GABAergic axons in the rat interpeduncular nucleus. *J Neurosci* 1993;13:2680–2688.

Léna C, Changeux JP. Role of Ca^{2+} ions in nicotinic facilitation of GABA release in mouse thalamus. *J Neurosci* 1997a;17:576–585.

Léna C, Changeux JP. Pathological mutations of nicotinic receptors and nicotine-based therapies for brain disorders. *Curr Op Neurobiol* 1997b;7:674–682.

Marler P, Terrace H. *The biology of learning*. Berlin: Springer-Verlag, 1984.

Mallet J, Huchet M, Shelanski M, Changeux JP. Protein differences associated with the absence of granule cells in the cerebella from the mutant weaver mouse and from X-irradiated rat. *FEBS Lett* 1974;46:243–246.

Merlie JP, Changeux JP, Gros F. Skeletal muscle acetylcholine receptor. Purification, characterization, and turnover in muscle cell cultures. *J Biol Chem* 1978;253:2882–2891.

Merlie, JP, et al. Synthesis of acetylcholine receptor during differentiation of cultures embryonic muscle cells. *Proc Natl Acad Sci USA* 1975;72:4028–4032.

Merlie J, Sanes JR. Concentration of acetylcholine receptor mRNA in synaptic regions of adult muscle fibers. *Nature* 1985;317:66–68.

Meunier JC, Sealock R, Olsen R, Changeux JP. Purification and properties of the cholinergic receptor from Electrophorus electricus electric tissue. *Eur J Biochem* 1974;45:371–394.

Mikoshiba K. Inositol 1,4,5 triphosphate (IP3) receptor. *Nippon Yakurigaki Zasshi* 2003;121:241–253.

Mikoshiba K, Huchet M, Changeux JP. Biochemical and immunological studies on the P 400 protein, a protein characteristic of the Purkinje cell from mouse and rat cerebellum. *Dev Neurosci* 1979;2(6):254–275.

Monod J, Changeux JP, Jacob F. Allosteric proteins and cellular control systems. *J Mol Biol* 1963;6:306.

Monod J, Wyman J, Changeux JP. On the nature of allosteric transitions: A plausible model. *J Mol Biol* 1965;12:88–118.

Mulle C, Changeux JP. A Novel type of nicotinic receptor in the rat central nervous system characterized by patch-clamp techniques. *J Neurosci* 1990;10:169–175.

Mulle C, et al. Existence of different subtypes of nicotinic acetylcholine receptors in the rat habenulo interpeduncular system. *J Neurosci* 1991;11:2588–2597.

Nachmansohn D. *Chemical and molecular basis of nerve activity*. New York: Academic Press, 1959;235.

Nikoshiba K. Inositol 1,4,5 triphosphate (IP_3) receptor. *Nippon Yakurigaki Zasshi* 2003;241–253.

Noda et al. Primary structure of alpha-subunit precursor of Torpedo californica acetylcholine receptor deduced from cDNA sequence. *Nature* 1982;299:793–797.

Numa S. A molecular view of neurotransmitter receptors and ionic channels. *Havey Lecture Series* 1989;83:121–165.

Olsen R, Meunier JC, Changeux JP. Progress in purification of the cholinergic receptor protein from Electrophorus electricus by affinity chromatography. *FEBS Lett* 1972;28:96–100.

Oswald RE, Changeux JP. Ultraviolet light induced labeling by non competitive blockers of the acetylcholine receptor from *Torpedo marmorata*. *Proc Natl Acad Sci USA* 1981;78:4430–4434.

Oswald RE, Changeux JP. Crosslinking of alpha-bungarotoxin to the acetylcholine receptor from Torpedo marmorata by ultraviolet light irradiation. *FEBS Lett* 1982;139:225–229.

Patrick J, et al. Molecular cloning of the acetylcholine receptor. *Cold Spring Harbor Symp Quant Biol* 1983;48:71–78.

Piette J, Bessereau JL, Huchet M, Changeux JP. Two adjacent MyoD1-binding sites regulate the expression of the acetylcholine receptor delta-subunit gene. *Nature* 1990;345:353–355.

Piette J, Klarsfeld A, Changeux JP. Interaction of nuclear factors with the upstream region of the alpha-subunit gene of chicken muscle acetylcholine receptor: Variations with muscle differentiation and denervation. *EMBO J* 1989;8:687–694.

Picciotto M, Zoli M, Léna C, Bessis A, Lallemand Y, Le Novère N, Vincent P, Merlo Pich E, Brûlet P, Changeux JP. Abnormal avoidance learning in mice lacking functional high-affinity nicotine receptor in the brain. *Nature* 1995;374:65–67.

Picciotto M, Zoli M, Rimondini R, Léna C, Marubio L, Merlo Pich E, Fuxe K, Changeux JP. Acetylcholine receptors containing 2-subunit are involved in the reinforcing properties of nicotine. *Nature* 1998;391:173–177.

Raftery MA, Hunkapiller M, Strader CD, Hood LE. Acetylcholine receptor: Complex of homologous subunits. *Science* 1980;208:1454–1457.

Ramon y Cajal. *Histologie du système nerveux de l'homme et des vertébrés*. Paris: Maloine, 1909–1911.

Redfern PA. Neuromuscular transmission in newborn rats. *J Physiol (Lond)* 1970;209:701–709.

Revah F, Bertrand D, Galzi JL, Devillers-Thiéry A, Mulle C, Hussy N, Bertrand S, Ballivet M, Changeux JP. Mutations in the channel domain alter desensitization of a neuronal nicotinic receptor. *Nature* 1991;353:846–849.

Revah F, Galzi JL, Giraudat J, Haumont PY, Lederer F, Changeux JP. The noncompetitive blocker ^3H chlorpromazine labels three amino acids of the acetylcholine receptor delta-subunit: Implications for the alpha helical organization of the MII segments and the structure of the ion channel. *Proc Natl Acad Sci USA* 1990;87:4675–4679.

Schaeffer L, de Kerchove A. and Changeux JP. Targeting transcription to the neuromuscular synapse. *Neuron* 2001;31:15–22.

Schaeffer L, et al. Implication of an Ets and Notch related transcription factor in synaptic expression of the nicotinic acetylcholine receptor. *EMBO J* 1998;17:3078–3090.

Sobel A, Weber M, Changeux JP. Large scale purification of the acetylcholine receptor protein in its membrane-bound and detergent extracted forms from Torpedo marmorata electric organ. *Eur J Biochem* 1977;80:215–224.

Steinlein O, et al. A missense mutation in the neuronal nicotinic acetylcholine receptor alpha 4 subunit is associated with autosomal dominant nocturnal frontal lobe epilepsy. *Nature Genet* 1995;11:201–203.

Von Economo C. *The cytoarchitectonics of the human cerebral cortex*. London: Oxford University Press, 1929.

Weber M, Changeux JP. Binding of Naja nigricollis ^{3}H-alpha-toxin to membrane fragments from Electrophorus and Torpedo electric organs. 1. Binding of the triatiated alpha-neurotoxin in the absence of effector. *Mol Pharmacol* 1974a;10: 1–14.

Weber M, Changeux JP. Binding of Naja nigricollis ^{3}H-alpha-toxin to membrane fragments from Electrophorus and Torpedo electric organs. 2. Effect of the cholinergic agonists and antagonists on the binding of the tritiated alpha-neurotoxin. *Mol Pharmacol* 1974b;10:15–34.

Weber M, Changeux JP. Binding of Naja nigricollis ^{3}H-alpha-toxin to membrane fragments from Electrophorus and Torpedo electric organs. 3. Effects of local anaesthetics on the binding of the tritiated alpha-neurotoxin. *Mol Pharmacol* 1974c;10:35–40.

Weill CL, McNamee MG, Karlin A. Affinity labeling of purified acetylcholine receptor from Torpedo californica. *Biochem Biophys Res Commun* 1974;61:997–1003.

Wiesel TN, Hubel DH. Single-cell responses in striate cortex of kittens deprived of vision in one eye. *J Neurophysiol* 1965;28:1060.

Zoli M, Le Novère N, Hill J, and Changeux JP. Developmental regulation of nicotinic receptor subunit mRNAs in the rat central and peripheral nervous system. *J. Neurosci.* 1995;15:1912–1939.