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From Muscle Endplate to Brain Synapses: A Short History of Synapses and Agonist-Activated Ion Channels

Review

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Introduction

This review gives a very personal view of some of the work that has led up to the present state of knowledge about synapses and ion channels. It would take a whole book to do justice to this topic, and we can only apologise in advance for failing to mention by name most of the people who have played an important part. Many of the "new" ideas that are proclaimed daily are actually quite old, and it is perhaps appropriate to remind the present generation of neuroscientists of how current views have evolved. A surprising number of them can be found in the prescient work of Bernard Katz and his colleagues at University College London, to whom this article is a tribute. Katz made fundamental contributions to both presynaptic (transmitter release) and postsynaptic (ion channel) aspects of synaptic transmission. We shall concentrate on the latter, and Erwin Neher will discuss presynaptic events in the next review.

The idea of a receptor originated in the nineteenth century, from the work of Paul Ehrlich and J. N. Langley. Langley studied the actions of atropine and pilocarpine, and in 1878 he noted (in the first volume of the *Journal of Physiology*, which he founded) that the inhibitory action of atropine could be overcome by increasing the dose of pilocarpine. Moreover, the restored response to pilocarpine could in turn be abolished by further atropine. Commenting on these results, Langley wrote:

> "We may, I think, without too much rashness, assume that there is some substance or substances in the nerve endings or [salivary] gland cells with which both atropine and pilocarpine are capable of forming compounds. On this assumption, then, the atropine or pilocarpine compounds are formed according to some law of which their relative mass and chemical affinity for the substance are factors."

If we replace *mass* by *concentration*, the second sentence can serve as well today as when it was written. Later, Langley (1905) coined the term "receptive substance" for what we would now call a nicotinic acetylcholine receptor. The first quantitative treatment of this problem was by A. V. Hill who, in 1909, gave the first derivation of the Langmuir equation (10 years before Langmuir). He derived the result in order to try to make sense of his results on the rate of action of nicotine and curari (sic) on nicotinic acetylcholine receptors (a problem, incidentally, that is still not entirely solved). A. V. Hill was, at the time, an undergraduate of Trinity College Cambridge, though he later held the Chair of Biophysics at University College London, in which job he was succeeded by Bernard Katz. Hill's ideas were developed over the next 30 years by A. J. Clark (e.g., Clark, 1933) and were extended to competitive antagonists at equilibrium by John Gaddum (e.g., Gaddum, 1937) and Heinz Schild (all three of whom held the Chair of Pharmacology at University College London).

There was, of course, no hint in 1878 that these observations had anything to do with synaptic transmission. That idea was developed by Henry Dale and his colleagues and was well established by the 1930s, through classical papers such as Dale, Feldberg, and Vogt (1936). This done, the way was paved for the study of agonist-activated ion channels, which had its roots in the 1940s and 1950s, although they were not called ion channels until later. Since then, we have learned how to measure the current flow through channels, we have learned much about the rates at which agonists bind and channels open and shut, and we know the primary structure of many channels and the shape of one of them (Unwin, 1995). But we still do not know in any detail how the function of a receptor relates to its structure, nor how exactly the agonist causes the structural rearrangement that is involved in opening and shutting.

Work on synaptic transmission in the brain, which had been pioneered by Eccles and others (see Eccles, 1964), underwent an enormous explosion after the introduction of brain slices and the patch clamp. We are now approaching the end of the "decade of the brain," and despite a great deal of interesting work and a lot of talk, it cannot honestly be said that there has been any great conceptual advance on the major issues; at the end of the decade, we not only still do not understand memory, but we still do not understand even long term potentiation. Perhaps it should have been a "century of the brain." What has been achieved is a considerable increase in the understanding of individual synapses in the brain. In some ways, they have turned out to be quite similar to the neuromuscular junction—very brief pulses of transmitter open postsynaptic ion channels, which depolarise or hyperpolarise the postsynaptic cell, and the time course of events is controlled largely by the rates of binding and opening of the receptor channel molecule itself. In other ways, though, central synapses are vastly more complicated.

Early Work on Synaptic Transmission in Muscle

The first estimate of the time course of transmitter action was derived from measurements of the rise and decay time course of the extracellularly recorded endplate potential and from double pulse experiments (Eccles et al., 1941). It was correctly inferred that the permeability change following a nerve impulse lasted only a few milliseconds.

The 1950s saw a rapid development in the understanding of the electrical events at synapses, in the wake of the elucidation of the action potential mechanism by

Hodgkin and Huxley in 1952. The discovery of the quantal release of acetylcholine from motor nerves by Paul Fatt and Bernard Katz (1950, 1951, 1952) quickly led to a better understanding of transmitter release. The nature of the change in permeability to ions that was caused by acetylcholine also gave rise to much discussion. Fatt and Katz (1951) had suggested that acetylcholine may "short-circuit" the membrane, "i.e. create aqueous channels through which small ions can pass without distinction," and "[i]t appears from these experiments that ACh raises both the Na and K conductance of the endplate and possibly also allows its permeability to other free ions" (quoted from del Castillo and Katz, 1956). The definitive demonstration of the nicotinic receptor as a nonselective cation channel was achieved by Takeuchi and Takeuchi (1960), in one of the first applications of the voltage-clamp method to the endplate.

The interpretation of ion channel behaviour entered a new era when del Castillo and Katz (1957) postulated the first realistic kinetic mechanism for agonist action (discussed in more detail below). In the same year, Katz and Thesleff (1957) used brief iontophoretic pulses of acetylcholine to the endplate for the first quantitative investigation of nicotinic receptor desensitisation. They concluded that a cyclic mechanism was needed to explain their observations, and almost every investigation of desensitisation since then has used some variant of their scheme.

Developments in Ideas about Receptors

Almost half a century passed after Hill's astonishing paper was published before the next critical development took place. This development was the recognition that the binding of an agonist to a receptor, and the subsequent activation of the receptor, had to be treated as two separate steps if any sense were to be made of the experimental results (Stephenson, 1956; del Castillo and Katz, 1957). Although this sounds like a small extension of what had already been done, it took a long time to come, and it proved extraordinarily fruitful (as discussed below). Indeed, the lessons of this development are still not universally appreciated.

The 1960s saw a continuous development of ideas about ion channels (reviewed by Rang, 1973; Colquhoun, 1975), and the first radioligand-binding experiments were also published (Paton and Rang, 1965). It was known that the start of the equilibrium concentration response curve for acetylcholine was sigmoid rather than Langmuirean (Katz and Thesleff, 1957; Jenkinson, 1960; Changeux and Podleski, 1968; Rang, 1971). This cooperativity had suggested that it was likely that two acetylcholine molecules were needed to open the channel, long before it was known that there were two α subunits. The early ideas of Wyman (see Wyman and Gill, 1990) had suggested the possibility that ligands might bind selectively to two preexisting conformations of a protein, so influencing the equilibrium between them. This idea was developed by Monod, Wyman, and Changeux (1965), in the context of cooperative enzymes, and it was soon applied to the acetylcholine receptor (Karlin, 1967; Colquhoun, 1973). This extension of the ideas of del Castillo and Katz (1957) gave a more concrete interpretation of how an agonist could open a channel.

The First Approach to Single Channels: Noise Analysis

In the early 1970s, the advent of noise analysis (Katz and Miledi, 1970, 1971, 1972) allowed the first (indirect) inferences about the properties of single ion channels. In the 1970 paper, Katz and Miledi showed fluctuations of the response to acetylcholine that were so obvious that one wondered why nobody had noticed them before. Katz and Miledi suggested, correctly, that the fluctuations were caused by the random opening and shutting of individual ion channels, and used the amplitude of the fluctuations to estimate that the elementary depolarisation elicited by ACh was about 0.3 μV in amplitude. For the first time, there was a firm estimate of the number of channels opened by a single quantum of ACh. Katz and Miledi (1971, 1972) went on to exploit the temporal characteristics of the noise to estimate the duration of elementary events, as well as their amplitude. By plotting the distribution of frequencies in the fluctuations as a power spectrum, they were able to conclude, for example, that the channel that appeared after denervation had a longer mean open time than the adult channel, and that carbachol produced briefer openings than ACh. They were also able to estimate the single channel conductance as being of the order of 100 pS, within a factor of 3 of the value subsequently found by single channel channel measurements. This was far bigger than some previous biochemical estimates had suggested, and it finally settled the long-running discussion about whether the receptor was a pore or a carrier.

Soon afterwards, it was shown that inhibition of acetylcholinesterase did not change the elementary event but that the duration of miniature synaptic currents was greatly increased (Katz and Miledi, 1973). They concluded that, under these conditions, rebinding of acetylcholine to its receptors dominated the slow diffusion of transmitter from the synaptic cleft.

Noise analysis did not, however, have sufficient resolution to solve a long-standing problem. It was pointed out by del Castillo and Katz in 1957 that the simplest conceivable mechanism for the activation of an ion channel consisted of an agonist-binding step followed by a conformation change (shut to open) step. It was impossible to resolve the rates of these two separate steps, and because of that it was impossible to interpret what the time constant(s) observed in noise analysis meant in molecular terms. All that could be done in the absence of evidence was to make plausible assumptions. Anderson and Stevens (1973) made the assumption, very plausibly at the time, that the agonist binding was very fast compared with the conformation change. In this case, the time constant from noise can be equated with the mean open lifetime of the channel. They showed that the time constant for the decay of synaptic currents was the same as the time constant inferred from acetylcholine-induced noise and inferred that the transmitter was present in the cleft for only a very short time, so the time course of the synaptic current was dictated

largely by the rate at which channels close in the absence of free agonist. This was correct (under their conditions at least), and subsequently a similar conclusion has been reached at many central synapses too. Anderson and Stevens described this time constant as the mean open lifetime of the channel, but it subsequently turned out that this is probably not right, though there was no way to tell at the time. The answer had to wait for the greatly improved resolution allowed by single channel recordings.

Single Ion Channels and the Patch Clamp

The development of noise analysis had made it natural to think in terms of single ion channels, which had been observed in artificial bilayers, but in the early 1970s nobody had seen one in a biological membrane. There was quite a large range of estimates for the size of the current that a single channel might pass and no knowledge of the shape of the elementary current event. It was these problems that guided the quest to measure single channel currents directly. These questions were frequently discussed in Katz's Department at University College London, and they seemed to me (B. S.) to be central questions in synaptic physiology. Although estimates of channel conductance from noise analysis turned out to be reasonably accurate, there were many other estimates (e.g., from biochemical measurements) that were wildly inaccurate. When they met again in Göttingen, Erwin Neher (who was recording single channels from doped bilayers) and B. S. decided to attempt measurements of elementary synaptic currents with patch pipettes. Earlier work with noise analysis had suggested that the elementary events in chronically denervated muscle fibres were somewhat smaller and had a severalfold longer duration than those in the subsynaptic membrane (Katz and Miledi, 1971; Neher and Sakmann, 1976a). It was therefore decided that this was a suitable preparation for an attempt to see single channels. Restriction of the measurement to a small patch of membrane 1–2 μm in diameter, with a seal resistance of about 100 MΩ, was sufficient to reduce the background noise to less than 1 pA at the necessary bandwidth. One of our very first attempts in Göttingen in spring 1975 showed square pulse-like events in patch pipette recordings from denervated frog muscle and assured us that we were on the right path. The results were published in the following year (Neher and Sakmann, 1976b). The next few years were spent in controlling possible artefacts, developing new and more convenient preparations, and increasing the seal resistance to several GΩ, making patches mechanically stable. These improvements allowed measurements at a higher time resolution, and in conditions where it was possible to control both voltage and the ion concentrations on both faces of the membrane (Hamill et al., 1981).

The advent of the ability to observe the behaviour of single ion channel molecules revolutionised the field, partly because it allowed observation of events around 10 times faster than could be achieved with methods used previously. Because single molecules behave in a random way, classical kinetic analysis could not be used for interpretation of observations, and an entirely new body of theory had to be developed to allow the interpretation of single molecule behaviour. The first general treatment of the problem was given by Colquhoun and Hawkes (1977). In fact, this paper was started before we knew about the work of Neher and Sakmann, and arose from my (D. C.) meeting Alan Hawkes in the University College common room in the late 1960s. His stochastic expertise, acquired in studies of road traffic flow, stimulated an interest (which, at the time, was entirely theoretical) in visualising what was happening at a one-molecule level (Colquhoun, 1970, 1971). The 1977 paper started life as a general description of noise and relaxations, with an attempt to relate the results to the underlying behaviour of single molecules. It was found that even with the fastest rate of agonist binding that is physically possible, it was still often the case that the time constant from noise was longer than the mean channel open time—i.e., it was not possible to attain the "fast-binding" limit. This was clear even for the simplest mechanism, that of del Castillo and Katz (1957). The paper was sent to Bernard Katz in January 1977 for his opinions (and because at that time papers for Royal Society journals had to be submitted via a Fellow). With characteristic perspicacity, he asked us to explain in more physical detail the reason for this discrepancy. It was only because of his comments that the figures (Figures 1E and 3D), which show channel activations as a burst of closely spaced openings and the distribution of the number of openings per burst, were added to the paper, though the single channel aspects of this work mostly came later (e.g., Colquhoun and Hawkes, 1982; Colquhoun et al., 1997).

In autumn 1977, we (D. C. and B. S.) met for the first time at a symposium at the Domaine de Seillac, a French chateau near Blois. During conversation, while we were watching a lengthy, and rather cold, demonstration of hunting trumpets, it emerged that bursts of openings had in fact been observed in Göttingen. This led at once to plans to test the theory, and the resulting papers (Colquhoun and Sakmann, 1981, 1985) gave a description of a plausible kinetic mechanism for the acetylcholine receptor. The results allowed the first estimates of all of the rate constants in the mechanism (and, less expectedly, also showed the existence of mono-liganded channel openings). Although the analysis has been refined and extended since then, the gist of the ideas seems, so far, to have stood the test of time.

The Relationship between New Methods and Old Problems

The significance of this work on ion channels extended far beyond the description of the acetylcholine receptor; it also impinged on a much older pharmacological problem. Stephenson (1956) had pointed out the very important fact that the action of an agonist could not be described by an affinity constant alone. In addition, some measure of the ability of the agonist to activate the receptor (i.e., in our case, to open the channel) was essential too (he termed the latter the *efficacy* of the agonist). This postulate was, very rightly, enormously influential (though widely misunderstood, as may be seen by the inaccurate accounts that are found in most

textbooks to this day). Stephenson had formulated his theory in a "black box" manner, but unfortunately it turned out subsequently that the box was too black, and it was not possible to measure the postulated quantities without knowing something about mechanism (Colquhoun, 1987). This stood in strong contrast to Schild's method for estimating the equilibrium constant for an *antagonist*, which is remarkably independent of the contents of the black box. Just after Stephenson's paper, del Castillo and Katz (1957), quite independently, addressed essentially the same problem. They, however, took the opposite approach, and discussed the problem in terms of a simple but specific mechanism, an agonist-binding step followed by a conformation change step. Their motive was very similar to Stephenson's, which was to explain the action of partial agonists. In their mechanism, the equilibrium constant for the second step provided a physical embodiment of Stephenson's efficacy. The analysis of single channels had allowed "efficacy" to be defined in a concrete way, and to be measured.

The advent of single channel measurements also resulted in a great improvement in the oldest of pharmacological measurements, the equilibrium concentration response curves. Sakmann, Patlak, and Neher (1981) showed that when high agonist concentrations were used, clusters of openings, separated by long silent periods in desensitised states, were observed. These clusters mostly originated from one channel, so the fraction of time for which one channel is open (P_{open}) could be measured directly. When P_{open} is plotted against agonist concentration, a (fairly) complete equilibrium concentration response curve can be obtained (e.g., Colquhoun and Ogden, 1988). This sort of curve has the enormous advantage over all earlier sorts of measurement that the response is measured on an *absolute scale*, not merely relative to some arbitrary maximum response. This method also allowed the effects of desensitisation to be eliminated, which is usually a great advantage because in most (though not all) cases desensitisation is more of an experimental nuisance than a physiologically interesting phenomenon. For example, attempts to measure the maximum response are often foiled by desensitisation.

The Binding-Gating Problem

Although the ideas of Stephenson and Katz are now quite old, they have assumed a new importance in the age of molecular biology. It is now commonplace for mutations to be made in a receptor protein with the aim of locating the position of the agonist binding site. But these old ideas make it obvious that the interpretation of such experiments may be far from obvious. Clearly the EC_{50} for the agonist (often, but unhelpfully, referred to as "apparent affinity") is affected by both steps in the reaction, the initial binding step and the conformation change step. Even now it is not uncommon to read that the properties of the binding site can be inferred by doing a ligand-binding experiment, whereas a glance at the simple model of del Castillo and Katz (1957) shows that this is obviously not true (for any ligand that causes a conformation change): the affinity for agonist as measured in a radioligand-binding experiment also depends on *both* steps in the mechanism. There is, of course, no reason to think that the mutated amino acid is in the binding site unless it is the first (binding) step that is affected rather than the second, and it is not at all easy to obtain evidence that this is the case. This problem, often referred to in the ion channel context as the "binding–gating problem," has been widely ignored, and it will be very interesting to see, when full structures become available, just how many mutation experiments have led to incorrect conclusions as a result. It will be equally interesting to see how much sense can be made of the relationship between protein structure and mechanism when the analysis is done properly. A more sophisticated version of the analysis used by Colquhoun and Sakmann has been used by Sine and Auerbach to interpret mutations of the nicotinic acetylcholine receptor that cause myasthenic syndromes (e.g., Sine et al., 1995). Their analysis provides a good illustration of the fact that the importance of considering mechanism extends far beyond an esoteric interest in kinetics—indeed, it is critically important for the sort of problems that recombinant DNA methods are now being used so widely to elucidate.

The Age of Molecular Biology

By the mid 1980s, it had become obvious that molecular biology was going to become enormously important in investigation of the structure and mechanism of ion channels. Shosaku Numa's laboratory in rapid succession cloned the genes of the ACh channel (see Numa, 1989), and a rather intense and enjoyable collaboration began to develop between physiology and molecular biology. Green, Heinemann, and Guisella review this enormous field elsewhere in this issue of *Neuron*.

Structure–Function Relations

The earliest studies on the acetylcholine receptor channel concentrated on structure and ion permeation. For example, it had been demonstrated that the receptor that is present in the membrane of denervated muscle fibres is different from that in normal adult endplate (Katz and Miledi, 1971). This type of receptor is also present in the endplate of neonatal muscles and is replaced by the adult receptor during postnatal development (Sakmann and Brenner, 1978; Fischbach and Schuetze, 1980). An early success for molecular biology was the demonstration that the difference between them resulted from replacement of the foetal γ subunit by the adult ϵ subunit, which was coded by a different gene (Mishina et al., 1986).

It was perceived, quite rightly it has turned out, that it was going to be easier to interpret the effects of mutations on the flow of ions though a channel *while* it was open, than to interpret the effects of mutations on ligand binding and gating (because these two effects are hard to separate, as mentioned above). For example, Imoto et al. (1988) showed a remarkably clear and direct relationship between the flow of ions through the channel (its conductance) and the number of negative charges in rings of charged residues at the inner and outer mouth of the channel (and in so doing, confirmed that the second transmembrane domain formed part of the channel lining and was orientated in the way suggested on the

basis of hydropathy plots). The relationship was simple because the channel conductance depended only on the total charge in these rings, but not on which subunit the charges were located.

Back to Muscle Function

A new line of research at the neuromuscular junction was triggered by the discovery that certain human diseases, the congenital myasthenic syndromes (see Vincent et al., 1997), are caused by mutations in the muscle nicotinic receptor. Some sophisticated analysis has been done on such mutants (e.g., Sine et al., 1995), and they have had a good deal of success in distinguishing between mutations that affect binding and those that affect gating. For every other sort of channel, apart from the muscle endplate, there is not enough knowledge of the kinetic mechanism for firm conclusions to be drawn, though this does not usually prevent them from being drawn anyway, and some of the conclusions will, no doubt, turn out to be right.

One of the next stages in analysis will often be to put the mutant into a genetically manipulated mouse. A first stage in this process was to knock out the nicotinic ϵ subunit. When this is done the mice become myasthenic, and the lack of the ϵ subunit is not compensated by the slight upregulation in the expression of the γ subunit, which normally is switched off after birth (Witzemann et al., 1996). Unfortunately, this does not answer the question of why the γ to ϵ switch has evolved, but the simple assumption that subunit redundancy will ensure synaptic function in case of disease does not seem to hold.

The Diversity of Subunits

One of the shocks of molecular biology was the revelation that many receptors are far more complex than had previously been assumed. The muscle nicotinic receptor is the simplest, but there are 11 different subunits for neuronal nicotinic receptors, at least 14 for the $GABA_A$ channel, and 8 for the non-NMDA (AMPA and kainate) channels, though glycine and NMDA channels seem to be a bit simpler. On top of this, there are many more variants that result from alternative splicing and RNA editing. The number of possible subunit combinations is, for many receptors, astronomical. It immediately became a major problem to discover (1) which subunits assemble together to form native receptors, and (2) whether the wide diversity of structures is useful to the organism. Progress has been slow on these two central problems. In many cases, we do not know the native composition with any accuracy, and even when we have a good idea about that, we often do not know whether that particular composition is essential for function or whether another would do just as well. In some cases, there are receptors present whose function is not yet known at all (see Sivilotti and Colquhoun, 1995). The problem of subtype specificity is, of course, not limited to ion channels. Similar questions have arisen about almost every protein that has been cloned. The answers are not only of intellectual interest, but they are also of vital importance for therapeutics. If a particular subtype has a well-defined function, then a drug that is selective for that subtype may be useful. This approach has been exploited successfully in the periphery (e.g., selective blockers for adrenoreceptor subtypes or histamine receptor subtypes), but has yet to come to fruition in the brain.

An extra layer of complexity is added by the fact there are many processes, such as phosphorylation, that are now known to regulate the activity of channels in experimental conditions, but in very few cases is it known whether such regulation matters much for the animal. A good example of the latter problem is again provided by the muscle nicotinic receptor. It is known that the rate of desensitisation of this receptor is altered by phosphorylation, but since there is very little reason at all to think that the receptor ever becomes desensitised under physiological conditions, it is unlikely that this phenomenon matters much.

Synapses in the Brain

The development of methods to record from cells expressing recombinant receptors, and to record from brain slices, triggered a new industry of investigations into synapses and ion channels. Many of these investigations basically followed the pattern that was developed at the neuromuscular junction. The aims have been to explain the time course of synaptic currents in terms of the underlying channel events and to investigate the function of the channels by making mutations. One aspect that is quite different from anything that was done at the neuromuscular junction has been the investigation of the very complex behaviour of two or more connected neurons.

One channel in the CNS that has been studied intensively is the NMDA type of glutamate receptor. NMDA receptors occur at most central glutamatergic synapses, but they are not the receptors that are responsible for the primary process of neurotransmission. They consist of a ubiquitous NR1 subunit (which has many splice variants), combined with one or more of the four NR2 subunits (NR2A–NR2D). They are certainly important, because mice in which the NR1 gene has been knocked out soon die. It is also true that their role in the normal physiology of the adult animal is not at all well understood (though this is rarely admitted). There is good reason to think that they have a role in the development of the nervous system, and if they are excessively activated they can certainly cause convulsions and kill cells. There is also no doubt that they play a role in long term potentiation (LTP) in some parts of the brain (though not in others), and for this reason there has been a great deal of speculation that they may have some role in memory, though the thesis that LTP is related to memory is still speculative. The properties of NMDA receptors are unlike those of any other agonist-activated channel. One most unusual characteristic is that it takes two agonists to open the channel, glutamate (bound to NR2 subunits) and glycine (bound to NR1 subunits). The channel is therefore reliant on the presence of ambient glycine, though the physiological significance, if any, of this odd property is not yet known. The other unusual property is that the channel spends most of its time blocked by extracellular magnesium, the block being relieved when the cell is depolarised (by other inputs).

Other channels (those activated by ACh, $GABA_A$, glycine, AMPA) are responsible for fast synaptic transmission, and a brief pulse of agonist causes a channel activation that, in most cases, consists of a compact burst of closely spaced openings that lasts for only a few milliseconds. In sharp contrast, exposure of an NMDA receptor to glutamate for one millisecond causes activations that last much longer (up to several seconds for an NR1/NR2D receptor). In contrast to endplate receptors, the NMDA channel is actually shut for most of the time that agonist is bound, the activation consisting of widely spaced openings spread over a long time.

The combination of long activations and voltage-dependent (and hence activity-dependent) block by magnesium means that the NMDA receptor must act as a sort of coincidence detector. Like most cation channels, the NMDA receptor channel is permeable to calcium, as well as to sodium and potassium, and its calcium permeability is somewhat greater than others. This fact, combined with the length of the channel activations, means that opening of NMDA channels allows a substantial influx of calcium. The consequent increase in intracellular calcium concentration is bound to affect many intracellular processes (and in excess certainly kills cells). These actions are well documented, but relating them to the physiology of a normal animal is a harder task.

The use of genetically manipulated animals has not yet given any clear idea about the physiological role of NMDA receptors. The fact that knockout of the NR1 or the NR2B subunit causes early death certainly strongly suggests an important function but tells us nothing about what it is. The knockout of the NR2A, NR2C, and NR2D subunits, in contrast, causes relatively little change in the behaviour of the animal (which is equally uninformative about their importance to the animal).

Conclusions and Problems

The ability to observe one (macro)molecule doing its business and the ability to change any chosen amino acid in that molecule are indeed exciting. But we are faced with thousands of possible channels and a million and one *potential* regulators of them. The observation of phenomena has far outstripped the knowledge of which of them are actually important for the animal. This is, perhaps, the biggest challenge for the future. The main hope for addressing the central question of the physiological role of receptors, and of their subtypes, has for some time been the use of genetically manipulated animals. The outcome of much effort has, up to now, been somewhat disappointing. In many cases the animal dies, so it is concluded that the gene in question is important (but we still do not know for what). In many more cases the effects of knockout are quite small (in which case the conclusion is often the same—the gene must be important because mechanisms must exist to compensate for its loss). Let us hope that switchable, and region-specific, knock-in of mutant receptors will be more informative.

These new and exciting methods, combined with more sophisticated physiological measurements, will no doubt lead to great advances. A first step is to recognise frankly what is not known. Perhaps every new postdoctoral student in neuroscience should be required to read one of Bernard Katz's papers each month. The clear and straightforward language, the lack of exaggeration and excessive speculation, and the frank admission of when things are not known stand as an example to all of us.

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