

CLASSICAL PERSPECTIVES

What have we learned from single ion channels?

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The only problem about being asked to write a perspective on the Colquhoun & Sakmann (1985) paper published in *The Journal of Physiology* is that I'm not yet sure whether the conclusions of that paper were quite right.

Up to 1985

The history of the events that led up to this paper has been described already so it won't all be repeated here (Colquhoun & Sakmann, 1998). Sakmann became interested in the idea that single channels might be observed directly while working in Bernard Katz's department at UCL in the early 1970s. When he returned to Göttingen to work with Erwin Neher, they soon achieved their aim (Neher & Sakmann, 1976). That momentous paper came out while Alan Hawkes and I were formulating a general theory of noise analysis, and it resulted in some last minute additions to the paper (Colquhoun & Hawkes, 1977). Up to that time, it had been supposed that synaptic current decay depended on the mean duration of openings of the individual ion channels. This can be true only if binding of the transmitter is much faster than other processes and our calculations seemed to indicate that this was a physical impossibility. The realization that it was now possible to observe single channels, and some prodding by Bernard Katz, led to the argument being put into terms of single molecules. In that form, the prediction was that, even when the transmitter binds as fast as is physically possible, it will activate not just one channel opening, but that the channel will re-open a few times before the agonist dissociates (Fig. 1). In other words, the event that underlies the synaptic current is actually a brief burst that consists of several openings separated by brief shuttings, though it wasn't until 1997 that the formal relationship between burst

length and synaptic current time course was elucidated (Colquhoun *et al.* 1997).

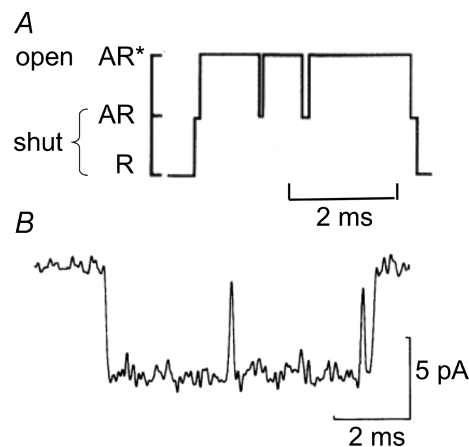
In the same year, 1977, I met Bert Sakmann and we set about testing the predictions. The channel openings looked surprisingly like the theoretical prediction (see Fig. 1). Figure 1A uses the mechanism proposed by del Castillo & Katz (1957). By the time we started work, the giga-ohm seal method had been discovered (though not yet published) so we could get sufficient resolution to see the brief shuttings that separate individual openings. Their mean duration was about 20 μ s for acetylcholine and 45 μ s for suberyldicholine, far too brief to be resolved in macroscopic measurements. Some preliminary results were published in *Nature* in 1981, but later a proper paper, the subject of this note, was finished in 1985. This being the time before the Research Assessment Exercise, it seemed quite normal to spend 7 years on it (Fig. 2). Now, that would probably get you fired.

Since Hawkes had by then developed a fairly complete theory of the burst behaviour of ion channels (Colquhoun & Hawkes, 1982) we had the equipment to interpret the results, though at that time there was no way to extract the whole of the information in the record. We had to look separately at distributions of open times, shut times, burst lengths, etc. It had been reported that successive open and shut times were negatively correlated (Jackson

et al. 1983) and the relevant theory just published (Fredkin *et al.* 1985) so we were able to exploit the information about connections between states that comes from measurement of correlations, albeit in a rather crude way. It seems quite surprising in retrospect that we were able to obtain tolerable estimates of rate constants even for quite complex models such as a receptor with two different binding sites and three open states. Astonishingly, the rate constants came out within a factor of two or so of the values that we'd guessed to calculate predictions in 1977, before a burst of openings had ever been seen. Single channels provided a far higher resolution than macroscopic measurements and the experiments seemed to provide the first solution of the binding-gating problem, which had previously been intractable. The results were interesting because they provided an insight into how ion channels, and hence synapses, work. They also had a separate interest to me as a pharmacologist, because they seemed to provide the first genuine measurements of affinity and efficacy (Colquhoun, 1998).

What's happened since 1985?

The experimental methods for recording channels have improved only marginally since 1985. The main technical advance

**Figure 1**

A, a typical predicted burst (in this case with 3 openings) based on the fastest binding that was physically feasible (taken as $5 \times 10^8 \text{ M}^{-1} \text{ s}^{-1}$). R represents the receptor and A the agonist (taken from Fig. 1, Colquhoun & Hawkes, 1977). B, an observed burst of openings (frog endplate, 100 nM suberyldicholine) (taken from Fig. 1, Colquhoun & Sakmann, 1985).

since then has been the development of better methods for fitting data. In 1990 an exact method was found to allow for the effect of missing openings or shuttings that are too short to be seen (Hawkes *et al.* 1990, 1992). That isn't a trivial problem: neglecting it can cause very large errors. In the 1980s, we had to use approximate retrospective corrections, but now it could be done properly. The way was opened to develop a maximum likelihood method that took into account the sequence of open and shut times (HJCFT program, www.dcsite.org.uk, http://barrel.med.buffalo.edu/wiki/index.php/main_page) and get optimal estimates for all the rate constants (up to 18 so far) directly, in a single operation. Two (free) computer programs are now available for doing the fitting, but nonetheless, attempts to interpret the fine structure of ion channels in terms of mechanisms has remained a minority pastime. Perhaps that is because it is the world's slowest method of generating an exponential. You need to measure many thousands of channel openings before the interesting bit begins. It's still much faster to determine an EC_{50} , even if it doesn't tell you what you want to know.

One of the main aims of the work when it was started was to make sense of structure–activity relationships. That has always been a central aim in pharmacology, though it has met with little success if you judge success by ability to predict. As the original work was going on, the revolution wrought by molecular biology was just

starting. By the end of the 1980s, another question had become commonplace: what happens if we change the structure of the receptor, rather than that of the agonist? In particular, what can mutations in the amino acid sequence of the receptor tell us about how the receptor works, and what are its functional domains. If you want to know where the agonist binding site is located on the receptor protein by mutating amino acids in candidate regions, you need to be able to tell whether the mutation has altered the ability to bind to the resting conformation of the receptor, or whether it has altered the ability of the receptor to change conformation in response to the binding. This binding–gating problem is, in principle, exactly the same as the older pharmacologists' problem of separating affinity (binding) and efficacy (Colquhoun, 1998). It was already clear in 1980 that this problem could not be solved either by measurements of agonist potency (changes in EC_{50}), or by ligand-binding measurements. The interpretation of fine structure that we proposed was accepted with an ease that is almost alarming. It has been used, with little change, ever since, for studies on mutant receptors, and on agonist structure–activity relationships (see, for example, Colquhoun *et al.* 2003; Mitra *et al.* 2005; Sine & Engel, 2006).

There is little doubt that the ability to measure the efficacy (gating) of an agonist that these methods provide is more or less right, and the ability to measure response–concentration curves on an absolute scale has proved to be useful.

It also has to be said that the aim of allowing predictions of the relationship between structure and activity, or the ability to predict the effect of a mutation, is still quite low. It is still the case that mutations that are undoubtedly far from the binding site appear to affect the binding affinity to the resting state (e.g. Colquhoun *et al.* 2003). The activation process seems to be more subtle than we had hoped in 1985. A possible reason for this is that, although we can measure the gating well, it may not be so easy to estimate the affinity for binding to the resting state. The sort of reaction mechanisms that were postulated in 1985 have changed remarkably little since. Any inferences you make about how an ion channel works are dependent on postulating a mechanism that is sufficiently close to physical reality. It could be that what was called 'affinity for the resting state' in 1985, and in almost every paper since, is actually the affinity for a molecule that has already changed conformation. If that were the case, a mutation anywhere in the molecule that affected the conformation change would appear to affect 'affinity'. In the case of the glycine receptor, the resolution is sufficiently good that it has been possible to fit observations with a mechanism that includes a shut state that is intermediate between resting and open (Burzomato *et al.* 2004). This is the first substantial change in the sort of mechanisms used to describe channels since 1985. Its ability to describe partial agonists and mutants is in the process of being tested. It remains to be seen if this sort of analysis can

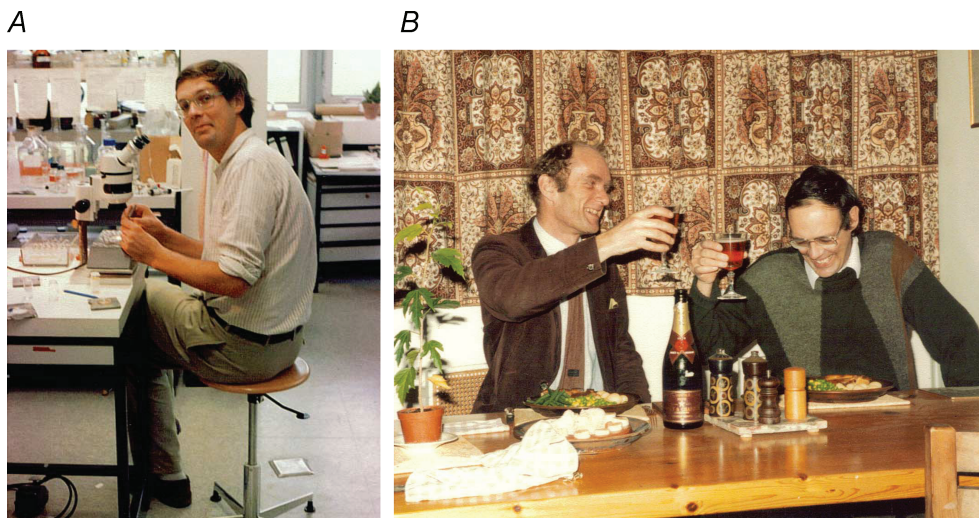


Figure 2

A, Sakmann dissects a cutaneous pectoris while D.C. patches, Göttingen, 1980. B, D.C. and B.S. at home, 11 pm, on 6 March 1985, back from UCL on the day the paper was finished.

yield estimates of affinity for the resting state that tell you about *only* the regions close to the binding sites.

Understanding the relationship between structure and function is still the holy grail, but it is taking even longer to find it than in Parsifal.

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