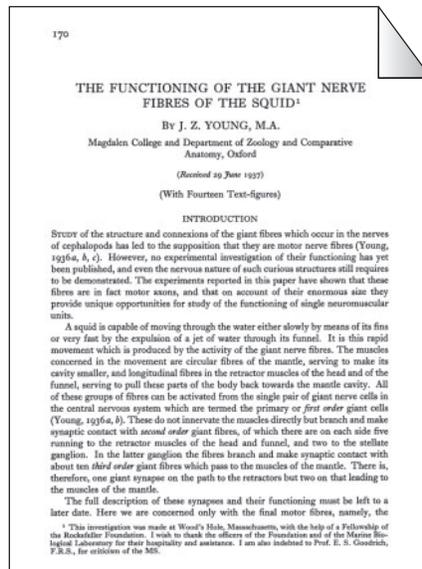


JEB Classics is an occasional column, featuring historic publications from *The Journal of Experimental Biology*. These articles, written by modern experts in the field, discuss each classic paper's impact on the field of biology and their own work. A PDF of the original paper accompanies each article, and can be found on the journal's website as supplemental data.

JEB CLASSICS

J.Z. AND THE DISCOVERY OF SQUID GIANT NERVE FIBRES



Richard Keynes writes about J. Z. Young's 1938 ground-breaking publication on the function of squid giant nerve fibres. A PDF file of Young's paper can be accessed as supplementary data at jeb.biologists.org

J.Z., to give him the title by which he was universally known, initially acquired an interest in cephalopods when working in Naples with Enrico Sereni in 1932 on the axons in the mantle connectives and stellar nerves of octopus. This led him to further studies at the Plymouth Marine Laboratory of some structures in the mantles of squid that he tentatively identified as giant nerve fibres (Young, 1936). In the summer of 1936 he visited Woods Hole in Massachusetts, determined to prove that these 'curious structures' were in fact motor axons. With F. O. Schmitt and R. Bear, he successfully examined the axoplasm of axons from the mantle of the squid *Loligo pealii* with polarized light, but failed in attempts with Ralph Gerard, Detlev Bronk and Keffer Hartline to make any oscilloscope recordings of action potentials from single fibres. However, he and Hartline did better one day when they found that application of a solution of sodium citrate to one end of the supposed axons generated a rhythmic discharge at the other, showing that they were indeed nerve fibres. He then made a careful study of the anatomy of the mantles, and in his classical paper on '*The functioning of the giant nerve fibres of the squid*' (Young, 1938), he showed that the third order giant axons served to bring about the precisely coordinated contraction of the mantle causing expulsion of a powerful jet of

water propelling the animals rapidly backwards or forwards according to the position of the funnel, sometimes accompanied by a slug of 'ink' to assist the animal's escape.

Having confirmed that the squid giant axons did conduct action potentials, and having with R. J. Pumphrey in 1938 (Young and Pumphrey, 1938) looked at the effect of their diameter on the rate of conduction, the only respect in which J.Z. subsequently involved himself in research on the ionic basis of conduction was to measure their electrolyte content (Young and Webb, 1945). He did, nevertheless, devote many years to an important series of observations at the Zoological Station in Naples on the mechanism of memory in octopus. And always interested in the animal as a whole he was working vigorously in the laboratory till the very end of his life on a wide range of problems. He will also be remembered as a teacher of great distinction, and as the author of two outstandingly wise and well-written textbooks on vertebrates and invertebrates.

It was, however, the introduction of giant nerve fibres by J.Z. that enabled the biophysics and biochemistry of excitable membranes to be properly studied in depth, which was said by Alan Hodgkin in 1973 to have done more for axonology than any other single advance in technique during the previous 40 years. J.Z. neatly summed up the impact that the discovery of giant motor axons would have on the field when he wrote '*on account of their enormous size [the squid's giant nerve fibres] provide unique opportunities for study of the functioning of single neuromuscular units*' (Young, 1938).

The first step in the exploitation of squid axons was taken in 1938 at Woods Hole by Kacy Cole and H. J. Curtis (Cole and Curtis, 1939) when they showed using external electrodes that during the passage of an impulse there was a rise and fall of the membrane conductance whose time course was very similar to that of the action potential. Then in the summer of 1939, both Curtis and Cole (1940, 1942) at Woods Hole, and Alan Hodgkin and Andrew Huxley (Hodgkin and Huxley, 1939) at the Laboratory of the Marine Biological Association in Plymouth, succeeded in slightly different ways in pushing long glass tubes, 0.1 mm in diameter and filled with K⁺ solutions, for some distance into the axons and thus recording the potential internally from an undamaged part of the membrane. To their great surprise they found that at the peak of

the conducted impulse the membrane potential did not, as was expected, fall close to zero, but was in fact substantially reversed.

After the end of six years of war that had interrupted biological research, the problem of accounting for the reversal of potential at the peak of the spike still remained unsolved. In writing up their 1939 experiments at greater length, Hodgkin and Huxley (1945) presented four elegantly argued alternative explanations, in none of which it was obvious that they had any faith. But then Alan Hodgkin dared to suggest that the permeability of the membrane to Na⁺ ions might undergo a transient increase. Working with squid giant axons at Plymouth in 1947, he and Bernard Katz were able to establish that the sodium theory was sound (Hodgkin and Katz, 1949). As has been described vividly by Hodgkin in his autobiography *Chance & Design* (Hodgkin, 1992), the great experimental triumph that came next was his and Huxley's development at Plymouth of the voltage-clamp technique for the quantitative analysis of the relationship between current and voltage in an excitable membrane (Hodgkin and Huxley, 1952).

There followed a series of research projects on related questions, for example the measurement of the net movements during the nerve impulse of sodium and potassium by Keynes and Lewis (1951); the establishment by Hodgkin and Keynes (1955a) of the existence of the sodium pump; studies by Caldwell, Hodgkin, Keynes and Shaw (1960) on the dependence of the sodium pump on a supply of phosphate-bond energy from ATP and arginine phosphate; the discovery of Hodgkin and Keynes (1955b) in Cambridge, using cuttlefish giant axons, of the manner in which K⁺ ions diffused in single file through the voltage-gated potassium channels in nerve membranes; and to crown Hodgkin's direct participation in experiments on squid axons, the development of a method for perfusing them with a variety of solutions after squeezing out the axoplasm as described by Baker, Hodgkin and Shaw (1962), in order to carry out further rigorous tests of the ionic theory.

During the 1960s and 1970s, experiments on squid giant fibres continued to occupy many axonologists, an advance of particular interest being the records made for the first time independently at Woods

Hole by Armstrong and Bezanilla (1974) and at Plymouth by Keynes and Rojas (1974), of the sodium gating current. The existence of such currents generated by the transmembrane movements of the charged gating particles had been predicted by Hodgkin, but they had not previously been recorded because of their small size relative to the ion currents. Then in 1984 Numa and his colleagues in Kyoto had succeeded, as described by Noda et al. (1984), in cloning the sodium channel gene of the electric eel, and soon the primary amino acid sequences of the voltage-gated sodium, potassium and calcium channels in a great many animals were known. What is more, it turned out that the channel proteins could readily be expressed in *Xenopus* oocytes, where their properties could conveniently be examined by the patch-clamping techniques first developed by Neher and Sakmann (1976). Research on these lines is now being vigorously pursued in many laboratories all over the world on the properties of ion channels gated not only by membrane potential, but also by other agents.

Such work could be regarded as the ultimate offspring of J.Z.'s introduction of giant axons to biologists, though few of its practitioners have ever seen a squid. But as a postscript it may be added that for technical reasons the time resolution obtained when voltage-clamping a squid giant axon is appreciably better than when voltage-clamping a patch of oocyte membrane, and for the best records yet made of the time course of the rise and fall of the sodium gating current in a squid axon the reader should refer to those obtained in the old-fashioned way by Keynes and Elinder (1998) and their colleagues.

10.1242/jeb.01405

Richard Keynes
Cambridge University
 rdk12@cam.ac.uk

References

Armstrong, C. M. and Bezanilla, F. M. (1974). Charge movement associated with the opening and closing of the activation gates of the Na channels. *J. Gen. Physiol.* **63**, 675-689.
Baker, P. F., Hodgkin, A. L. and Shaw, T. I. (1962). The effects of changes in internal ionic concentrations on the electrical properties of perfused giant nerve fibres. *J. Physiol. Lond.* **164**, 355-374.
Caldwell, P. C., Hodgkin, A. L., Keynes, R. D. and Shaw, T. I. (1960). The effects of injecting 'energy-rich' phosphate compounds on the active

transport of ions in the giant axons of *Loligo*. *J. Physiol. Lond.* **152**, 561-590.
Cole, K. S. and Curtis, H. J. (1939). Electrical impedance of the squid giant axon during activity. *J. Gen. Physiol.* **22**, 649-670.
Curtis, H. J. and Cole, K. S. (1940). Membrane action potentials from the squid giant axon. *J. Cell. Comp. Physiol.* **15**, 145-157.
Curtis, H. J. and Cole, K. S. (1942). Membrane resting and action potentials from the squid giant axon. *J. Cell. Comp. Physiol.* **19**, 135-144.
Hodgkin, A. L. (1992). *Chance & Design. Reminiscences of Science in Peace and War.* Cambridge: Cambridge University Press.
Hodgkin, A. L. and Huxley, A. F. (1939). Action potentials recorded from inside a nerve fibre. *Nature* **144**, 710-711.
Hodgkin, A. L. and Huxley, A. F. (1945). Resting and action potentials in single nerve fibres. *J. Physiol. Lond.* **104**, 176-195.
Hodgkin, A. L. and Huxley, A. F. (1952). A quantitative description of membrane current and its application to conduction and excitation in nerve. *J. Physiol. Lond.* **117**, 500-544.
Hodgkin, A. L. and Katz, B. (1949). The effect of sodium ions on the electrical activity of the giant axon of the squid. *J. Physiol. Lond.* **108**, 37-77.
Hodgkin, A. L. and Keynes, R. D. (1955a). Active transport of cations in giant axons from *Sepia* and *Loligo*. *J. Physiol. Lond.* **128**, 28-60.
Hodgkin, A. L. and Keynes, R. D. (1955b). The potassium permeability of a giant nerve fibre. *J. Physiol. Lond.* **128**, 61-88.
Keynes, R. D. and Elinder, F. (1998). On the slowly rising phase of the sodium gating current in the squid giant axon. *Proc. R. Soc. Lond. B* **265**, 255-262.
Keynes, R. D. and Lewis, P. R. (1951). The sodium and potassium content of cephalopod nerve fibres. *J. Physiol. Lond.* **114**, 151-182.
Keynes, R. D. and Rojas, E. (1974). Kinetics and steady-state properties of the charged system controlling sodium conductance in the squid giant axon. *J. Physiol. Lond.* **239**, 393-434.
Neher, E. and Sakmann, B. (1976). Single-channel currents recorded from membrane of denervated frog muscle cells. *Nature* **260**, 799-802.
Noda, M. et al. (1984). Primary structure of *Electrophorus electricus* sodium channel deduced from cDNA sequence. *Nature* **312**, 121-127.
Young, J. Z. (1936). The structure of nerve fibres in cephalopods and Crustacea. *Proc. R. Soc. Lond. B* **121**, 319-337.
Young, J. Z. (1938). The functioning of the giant nerve fibres of the squid. *J. Exp. Biol.* **15**, 170-185.
Young, J. Z. and Pumphrey, R. J. (1938). The rates of conduction of nerve fibres of various diameters in cephalopods. *J. Exp. Biol.* **15**, 453-466.
Young, J. Z. and Webb, D. A. (1945). Electrolyte content and action potential of the giant nerve fibres of *Loligo*. *J. Physiol. Lond.* **98**, 299-313.