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EDITORIAL

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Invertebrate studies and their ongoing contributions to neuroscience

Abstract Invertebrates have been deployed very successfully in experimental studies of the nervous system and neuromuscular junctions. Many important discoveries on axonal conduction, synaptic transmission, integrative neurobiology and behaviour have been made by investigations of these remarkable animals. Their advantages as model organisms for investigations of nervous systems include (a) the large diameter of neurons, glia and muscle cells of some invertebrates, thereby facilitating microelectrode recordings; (b) simple nervous systems with few neurons, enhancing the tractability of neuronal circuitry; and (c) well-defined behaviours, which lend themselves to physiological and genetic dissection. Genetic model organisms such as *Drosophila melanogaster* and *Caenorhabditis elegans* have provided powerful genetic approaches to central questions concerning nervous system development, learning and memory and the cellular and molecular basis of behaviour. The process of attributing function to particular gene products has been greatly accelerated in recent years with access to entire genome sequences and the application of reverse genetic (e.g. RNA interference, RNAi) and other post-genome technologies (e.g. microarrays). Studies of many other invertebrates, notably the honeybee (*Apis mellifera*), a nudibranch mollusc (*Aplysia californica*), locusts, lobsters, crabs, annelids and jellyfish have all assisted in the development of major concepts in neuroscience. The future is equally bright with ease of access to genome-wide reverse genetic technologies, and the development of optical recordings using voltage and intracellular calcium sensors genetically targeted to selected individual and groups of neurons.

In the 1930s, studies on praying mantids by KD Roeder, on crustaceans by CL Prosser and on caterpillars by ED Adrian (1934) showed that isolated nerve cords or ganglia display inherent neural activity in the absence of sensory input, adding a new dimension to the predominant view of the time that nervous systems were complex stimulus–response machines. Roeder's work on mantids was also the first to demonstrate the importance of the release of inhibitory input on intrinsic activity in nervous systems, which is also fundamental to the understanding of motor systems of vertebrates.

A major advance in neuroscience was the demonstration that action potentials result from the gated activities of distinct ionic conductances (see Hodgkin 1964). JZ Young discovered that large 'spaces' in sections of squid (*Loligo forbesi*) nervous tissue were not, as previously assumed, blood or lymph vessels, but were instead 'giant' axons. This allowed the first intracellular recordings from neurons and enabled the groundbreaking voltage-clamp experiments by KC Cole, AL Hodgkin, AF Huxley, and B Katz (Hodgkin and Huxley 1952; cf. also Hodgkin 1964; Katz 1966). Hodgkin and Huxley, together with JC Eccles, were awarded the 1963 Nobel Prize for their classic work in this field. The work of Katz (1966), who undertook pioneering work on squid axons and synapses as well as vertebrate preparations, was acknowledged with the award of the 1970 Nobel Prize for work on the storage, release and inactivation of neurotransmitters.

Before HK Hartline's recordings from single cells in the eye of the invertebrate Limulus, which demonstrated the phenomenon of lateral inhibition, it was not generally believed that recordings from single neurons could yield useful information on the workings of the nervous system. In view of the large numbers of neurons in the vertebrate CNS, this is easy to understand, yet the subsequent demonstrations of columnar organisation in several brain regions, and the charting of visual processing from the retina to the primary visual cortex were developments which built on groundbreaking invertebrate studies and have amply justified the single cell approach.

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In the early 1960s, J Dudel and SW Kuffler demonstrated presynaptic inhibition using a crayfish preparation. The Kuffler laboratory at Harvard was hugely influential and this was where Otsuka et al. (1966) used the lobster to demonstrate release of the neurotransmitter GABA from inhibitory nerves. The 1960s also saw a renewed interest in the large diameter molluscan, crustacean, annelid and insect neurons, first described in many cases by nineteenth century anatomists. These were extensively exploited using the intracellular saline-filled glass microelectrode recording techniques. This led to an explosion of studies exploring the functions and interactions of identifiable neurons. The potentials offered by these techniques were captured in a key text on the structure and function of invertebrate nervous systems (Bullock and Horridge 1966). Using the leech, Hirudo medicinalis, Kuffler and colleagues demonstrated an important role for glial cells in controlling the environment of neurons. J Nicholls, DA Baylor and colleagues investigated the large neurons of the leech and demonstrated regeneration of specific neuronal connections (Baylor and Nicholls 1971). J Kehoe, P Ascher, GA Kerkut and others deployed molluscan neurons to great effect. E Kandel's exploration of the siphon withdrawal circuit of the mollusc *Aplysia californica* (Kandel and Schwartz 1982) made groundbreaking inroads into explaining behaviour in cellular terms, resulting in the award to E Kandel of the 2000 Nobel Prize for Medicine for discoveries concerning signal transduction in the nervous system. Simultaneous intracellular recordings with paired electrodes were also used by M Burrows and G Hoyle on locusts, gaining insights into neural networks, while the work of Burrows revealed the importance of non-spiking interneurons in the control of motor output. A detailed description of functional interactions in the crustacean stomatogastric ganglion was provided by A Selverston and colleagues.

Invertebrate models have also been used in the analyses of complex behaviour, notably the work by K von Frisch, R Menzel and others on honeybees. The mechanisms of olfactory learning in Drosophila are being unravelled at the cellular and molecular level, revealing that memory-related genes in Drosophila have conserved functions in mammals. Mutants such as *dunce* and *rutabaga* have been particularly instructive in understanding the molecular basis of memory (Davis 2005). Recently, C Bargmann and colleagues have identified a point mutation in a neuropeptide Y receptor in *Caenorhabditis elegans* that determines whether animals exhibit social or solitary feeding behaviour (de Bono and Bargmann 1998).

Biochemical studies, followed by molecular approaches, culminated in the sequencing of entire invertebrate genomes, first the genomes of *C. elegans* and *Drosophila melanogaster* but with many more added since. The application of forward and reverse genetics technologies, together with the widespread use of reporter genes (Brand and Perrimon 1993; Prasher 1995) has facilitated the assigning of function to molecular components involved in neural signalling. Gene silencing by RNA interference (Fire et al. 1998; Buckingham et al. 2004) and the use of microarrays (Kim et al. 2001) have been particularly fruitful areas in recent years and genome-wide molecular searches can now be undertaken (cf. Kamath and Ahringer 2003; Simmer et al. 2003).

What about the future? Although, at first sight, appearing to offer little to the classical neurophysiologist, the fruit fly *D. melanogaster* and the nematode, *C. elegans* are key organisms in a new wave of invertebrate neuroscience. Studies on Drosophila, illustrated by the work of Bate et al. 1981, show how this organism has led to an understanding of the roles of key genes in nervous system development, while the availability of learning mutants has allowed Davis (2005) and others to describe key genes participating in learning and memory. At the same time, the extremely simple nervous system of *C. elegans* (just 302 neurones in the adult hermaphrodite) provides a tempting lure for systems neuroscientists. Although there are a few papers describing its electrophysiology, the worm's nervous system offers few advantages that attracted neuroscientists to snails and insects. The neurons are small, and safely shielded from the electrophysiologist's microelectrode by a tough external cuticle. However, some of these difficulties are being overcome using new optical methods of recording. Optical techniques open up new possibilities; real-time imaging of calcium transients in identified chemosensory neurones has already been accomplished (Kerr et al. 2000). Recently, high-resolution, voltage-sensitive optical recording at the microsecond time scale has been demonstrated using a FRET approach involving membrane-bound GFP (Chanda et al. 2005). Given the availability of a plethora of strains with specific neurons or neuron classes labelled with GFP, this makes it possible, at least in theory, to record the activity of a high proportion of neurons in the worm nervous system.

The kind of contribution invertebrates are now making to neuroscience is changing. Traditional invertebrate models, with their large, accessible cells and well-partitioned nervous systems, will continue to supply insights into function. But these investigations will be complemented by genome-wide approaches applied to systems biology and to understanding human nervous system and neuromuscular disorders. Although intense research efforts have been directed at understanding neurodegenerative diseases, for example, comparatively little is known about the molecular pathways underlying many of them. This has made forward and reverse genetics approaches using invertebrate models particularly appealing (Culetto and Sattelle 2000; Kretzschmar 2005). Thus, contributions from invertebrate studies are likely to continue to play a major role in neuroscience.

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