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Invertebrate models of behavioural plasticity and human disease

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Abstract

The fundamental processes of neural communication have been largely conserved through evolution. Throughout the last century, researchers have taken advantage of this, and the experimental tractability of invertebrate animals, to advance understanding of the nervous system that translates to mammalian brain. This started with the inspired analysis of the ionic basis of neuronal excitability and neurotransmission using squid during the 1940s and 1950s and has progressed to detailed insight into the molecular architecture of the synapse facilitated by the genetic tractability of the nematode *Caenorhabditis elegans* and the fruit fly *Drosophila melanogaster*. Throughout this time, invertebrate preparations have provided a means to link neural mechanisms to behavioural plasticity and thus key insight into fundamental aspects of control systems, learning, and memory. This article captures key highlights that exemplify the historical and continuing invertebrate contribution to neuroscience.

Keywords

Invertebrate neuroscience, learning, memory, neurodegeneration, cephalopods, *Caenorhabditis elegans*, *Drosophila*, leech, molluscs, gastropods

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Introduction

The defining features of a nervous system, as opposed to a collection of secretory cells, is the capability for fast and directed signalling via synaptic contacts, which may be electrical or chemical: its evolutionary origin, or origins, continues to encourage much debate (Schmidt-Rhaesa et al., 2016). The simplest nervous systems, for example in cnidarians such as jellyfish, typically consist of a radial nerve net with no central control system, though some cnidarians show the beginnings of cephalisation, that is, groups of neurons to control specific tasks such as swimming. More complex bilateral animals, with a nerve cord and ganglia to provide control for each segment of the animal, arose later. These animals exhibit cephalisation in which, sensory ganglia are concentrated at the anterior of the animal. While the nervous systems of invertebrates evolved increasingly sophisticated morphological and organisational features, at the fundamental level of communication, many features of nervous systems were highly conserved (Walker et al., 1996). For this reason, research using invertebrate animals has made a significant contribution to our understanding of basic neuroscience that translates to the human situation.

Arguably, most notable was the analysis of the ionic basis of the resting membrane potential, action potential, and synaptic transmission in the squid giant axon and synapse (Hodgkin, 1964) which paved the way to an understanding of ion channels and their regulation of neural excitability and synaptic transmission, recognised by a Nobel Prize in 1963. There are also similarities between chemical neurotransmitters and their receptor transduction pathways throughout the animal phyla including acetylcholine (ACh), 5-hydroxytryptamine (5-HT, serotonin), catecholamines, and amino acids (Walker et al., 1996). For example, GABA was first identified in peripheral axons of crustaceans, and the groundbreaking work of Stephen Kuffler and colleagues led to its recognition as a major inhibitory neurotransmitter (Florey, 1991). This discovery was pivotal in the identification of other amino acids as transmitters, including glutamate and glycine. Invertebrates have also informed understanding on neuropeptides as transmitters and neuromodulators a good example being the discovery of the FMRFamide-like neuropeptides in molluscs which provided a route to discovery of mammalian neuropeptides with diverse physiological roles (Walker et al., 2009). At a neuronal circuit level, there is also homology, for example, between the insect central complex and the vertebrate basal ganglia (Strausfeld and Hirth, 2013). Over the past 50 years, a number of invertebrate animals have been utilised as informative model systems that, in addition to reducing the need to work on higher animals in concordance with the 3Rs principles, has facilitated the pace of discovery for mammalian neuroscience.

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Learning and memory in molluscs

The molluscan contribution to an understanding of learning and memory was fundamental: investigations conducted by a number of groups from the 1960s and 1970s, in particular utilising the gastropod sea hare *Aplysia californica*, established a core role for synaptic plasticity in the learned behavioural output of the animal (Castellucci et al., 1970; Sweatt, 2016). This insightful work secured another Nobel prize for invertebrate research (to Carlsson et al. in 2000 for '*Signal transduction in the nervous system*'). The particular advantage of working with gastropods, including snails, is that they have large central neurons which can be identified from preparation to preparation and which have been partially mapped by electrophysiological analyses (Benjamin, 2012; Frazier et al., 1967; Kerkut et al., 1975). Since the studies on *Aplysia*, the pond snail *Lymnaea stagnalis* and other snails have been widely used to study the neuronal mechanisms of associative learning including sensory-dependent mechanisms of memory lapse during consolidation of long-term memory (Marra et al., 2013). This invertebrate system has also been deployed more recently as a model for the investigation of memory loss in neurodegenerative disease by assessing the impact of amyloid-β1-42, a component of the plaques found in human brain in Alzheimer's disease, on retention of long-term memory (Ford et al., 2015). Following training, amyloid-β1-42 was injected into the snail and found to impair memory after 24 h but had no effect on memory retention if injected prior to training, suggesting an effect on memory maintenance but not on acquisition.

For further information on invertebrate learning and memory, the reader is referred to Menzel and Benjamin (2013) which includes research on a wide range of invertebrates including gastropods and cephalopods and further seminal studies on bees. It is noteworthy that these studies often illustrate that invertebrates have a remarkable, and often an under-appreciated, capacity for learning. Particularly noteworthy in this regard are studies on cephalopods, such as the octopus, *Octopus vulgaris*, which have complex nervous systems with around 500 million neurons. These have been deployed in training paradigms to investigate fundamental aspects of learning and memory and include studies illustrating a capacity for observational and social learning (Young, 1965; Zarrella et al., 2015).

Control circuits in the leech and decapod Crustacea

The ability to map, define and record from discrete circuits that underpin defined behaviours in invertebrates has provided a means to unravel fundamental principles of neural circuit organisation and provide insight into how the control systems of more complex higher animals may be organised. Here examples are provided from the leech, *Hirudo medicinalis* and from decapod Crustacea.

The nervous system of *H. medicinalis* is composed of a head ganglion, 21 segmental ganglia and 7 fused tail ganglia, with each segmental ganglion containing ≈400 neurons (Nicholls and Baylor, 1968). Cardiovascular function in the leech involves two myogenic heart tubes which are regulated in a coordinated pattern by a defined neural network to provide circulation. Analysing the relative role of pacemaker neurons and emergent features of the neural network can translate to control circuits in higher animals (Wagenaar, 2015) for example by providing a framework for understanding autonomic control systems. The locomotor behaviour of the leech has also been of interest: it can switch between crawling and swimming, and it has been shown that one higher-order decision-making command neuron is required for the intersegmental coordination of locomotor behaviour, again providing a model for understanding network regulation of behaviour.

In decapod Crustacea, two specific ganglia have been investigated in order to understand circuit function, viz, the cardiac ganglion (Maynard, 1955) and the stomatogastric ganglion (Selverston et al., 1976) of the lobster *Homarus americanus.* Both of these systems have provided insight into how neurons can deliver pacemaker activity and central pattern generation and have delineated ion conductances that underpin the neural pattern of activity (Cooke, 2002). In addition, the study of the stomatogastric ganglion has revealed roles for modulatory input from many neuropeptides, biogenic amines, and amino acids which come from neurons projecting into the ganglion, from sensory neurons and from neurohormones. Evidence suggests that vertebrate neurons and neuronal circuits are multifunctional like invertebrate circuits and so the latter's circuits can act as models for the more complex and less accessible vertebrate circuits (Nusbaum and Beenhakker, 2002).

Model genetic organisms, *Caenorhabditis elegans* and *Drosophila melanogaster*, molecular substrates of circuit function and disease

While the invertebrates discussed above have all been exceptionally informative in relating circuits to function, they have lacked the genetic tractability to drill down to the molecular underpinnings. In this respect, the model genetic animals, the nematode *Caenorhabditis elegans* and the fruit fly *Drosophila*, have come to the fore.

The bacteriovore nematode, *C. elegans* was introduced as a research tool by Professor Sydney Brenner (1974) and has been adopted world-wide with thousands of researchers making use of its experimental tractability. It was the first animal to have its genome sequenced; there are over 19,000 genes, with an estimated 38% of the protein coding genes having predicted orthologues in humans. For neuroscientists, it has the wonderful attribute of a simple nervous system, just 302 neurons in the hermaphrodite, for which circuits underpinning behavioural outputs have been mapped www.wormatlas.org (De Bono and Maricq, 2005). Early genetic screens identified hundreds of mutants that exhibited altered locomotory behaviours, feeding and egg-laying. Each of these were designated a three letter prefix and a number, *unc, eat* and *egl*, respectively. In subsequent years, the genetic bases of these aberrant behaviours was ascribed to mutations in specific genes and this provided a powerful and unbiased approach which led to the discovery of a large number of the key synaptic proteins required for neurotransmitter synthesis and storage, SNARE complex formation, neurotransmitter release and receptor transduction. Identification of mammalian orthologues of the *C. elegans* genes, for example, the mouse orthologues of the *uncs* the so-called muncs, yielded molecular insight into the evolutionary conservation of synaptic signalling.

The fruit fly *Drosophila* has served as a genetic model system for over a century (www.flybase.org.), and through the use of automated 3D imaging of the brain, this is now combined with availability of a connectome (Lin et al., 2015). In a similar fashion to *C. elegans*, the fly has provided insight into fundamental aspects of neural signalling, for example, the role of synaptotagmin as a calcium sensor for fast synaptic transmission (Littleton et al., 1994). Early research using *Drosophila shaker* mutants also made important contributions to molecular genetic analyses of neuronal excitability when it was shown these genes encode potassium channels (Salkoff et al., 1992). The functions of many presynaptic proteins have been studied using *Drosophila*, and these have been recently reviewed (Bellen et al., 2010).

Both *C. elegans* and *Drosophila* have been used as models for the study of human diseases, since they express homologues of many of the genes associated with human diseases including Alzheimer's disease, Duchenne muscular dystrophy, Parkinson's disease and autism spectrum disorder (Calahorro, 2015; Culetto and Sattelle, 2000; Wu and Lloyd, 2015). Transgenesis has permitted manipulation of the worm and fly to express human disease-related proteins including amyloid-β, tau, huntingtin, parkin and neuroligin and assess their impact using molecular, cellular and behavioural analyses. These models provide uniquely experimentally tractable systems for the study of neuron-specific toxicity and aberrant behaviours associated with the expression of human disease proteins. For example, a *Drosophila* tauopathy model exhibits microtubule destabilisation, axon transport disruption, synaptic defects and impairment of behaviour which can be ameliorated with a microtubule-stabilising peptide, NAPVSIPQ (Quraishe et al., 2013). *Drosophila* has also been pivotal in delivering insight into the neural regulation of biological rhythms and led to the discovery of the circadian genes including *period, clock, timeless* and *cycle* (Sehgal et al., 1994).

Summary

The discussion above has briefly outlined how the study of the nervous systems of invertebrates has, by virtue of the evolutionary conservation of fundamental signalling mechanisms, facilitated the pace of understanding the complexities of neural function in higher animals including human. Early studies made great advantage of the ability to record electrophysiologically from multiple neurons in defined circuits and to relate this to behavioural outputs. Latterly, the wealth of genetic tools and information for the nematode *C. elegans* and fruit fly *Drosophila* further increased the rate of knowledge acquisition. For neuroscience, this increase in knowledge has not been the sole benefit: invertebrates have also provided platforms for the discovery and development of new experimental tools and this continues apace, for example, by the development of optogenetics and most recently sonogenetics as methods for non-invasive control of neural activity (Husson et al., 2013; Ibsen et al., 2015).

While a mammalian centric view might highlight the opportunity to use invertebrates to model human disease, it should not be forgotten that the neurobiology of the invertebrates, is also important in its own right. There is a wealth of invertebrate behaviours, not touched on here, which are of increasing interest in terms of understanding sensory, inter-organism and inter-species signalling. Recent studies are revealing that even simple nematode worms use a complex array of pheromones to influence the behaviour of other animals in their group in a manner that enhances their fitness (Diaz et al., 2014). Moreover, invertebrates, particularly termites, bees, wasps and ants can live in complex social groups where individual animals have specific tasks within the society. The ability of invertebrates to orientate, navigate and migrate across thousands of miles is also a fascinating and, as yet, poorly understood phenomenon (Reppert et al., 2010).

Finally, these animals are core elements of our ecosystem, some beneficial others detrimental to our health, food security and environment. Understanding the neural basis of their behaviour, therefore, is of increasing importance in the 21st century, as the planet experiences the challenges of feeding and maintaining the well-being of an ever expanding population.

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