The contribution of invertebrates to the understanding of the vertebrate nervous system, its mechanisms, functions and pathological dysfunctions.

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The human brain and the nervous system are the immediate result of several (6-7) million years of hominid evolution, and ultimately of organismic evolution as they bear many similarities to the brain and nervous system of vertebrates. We readily accept that all vertebrate organisms such as fish, amphibians, reptiles, birds and mammals, share many anatomical features and major organs corresponding to our human organism: they have guts and intestines; they possess a circulatory system with a heart, kidneys and an excretory system. Less well accepted, perhaps, is the fact that these animals have brains and nervous systems which are also related to us. Novel analyses of the nervous systems of vertebrates allow retracing the occurring evolutionary changes with unprecedented precision using new technical tools from molecular biology, immunocytochemistry or molecular genetics. These analyses reveal the evolution of particular brain structures in new environments and their relation to different life styles, something that biologists call an “ecological niche”. We readily accept that primates like chimpanzees have many similarities in their body shape and behaviour to us, namely because they resemble to ourselves in many aspects. We even extend this judgement to our pet animals, dogs and cats, which exhibit some similarities to certain aspects of our own behaviour. Yet, we actually may be reluctant to grant such similarities to the brains of fish and frogs, despite the fact that these animals belong to vertebrates and exhibit the so-called “common vertebrate Bauplan”.

If similarities appear difficultly to most people when thinking about the nervous system of humans and that of amphibians or fishes, what could be said about the nervous system of invertebrates? Animals without backbones, the “squishes and crunchies” so to speak, appear as so utterly distant from us, that the question of similarity may even not be raised. Many people may even ask if creatures like flatworms, earthworms, snails and mussels, and all insects and crustaceans, have brains or nervous systems at all. Yet, all these animals feed, move and reproduce, thus revealing the presence of a nervous system responsible of their different behaviors. This simple consideration indicates that preconceptions in the case of invertebrates are not justified. As this argument may appear simplistic to detractors of invertebrates, we discuss here a series of arguments and evolutionary facts that may serve as an agenda to appreciate the enormous potential of invertebrates for research and understanding of the nervous system, its function and pathological dysfunctions.

The origin of the nervous system has been a question that haunted (and still haunts!) zoologists since a long time. With the advent of new molecular techniques, some answers to this particular question have been obtained. One feature that appears obvious is that all vertebrates, including humans, and most invertebrates, are in general bilaterally symmetrical and belong, therefore, to the Bilateria. Although many asymmetries have occurred during evolution so that some organs are only found on one side of the body (e.g. the liver), or in the nervous system the left and right hemispheres have specialized to serve different functions, the common organization plan is bilaterally symmetric. In addition, the whole body is separated into different portions or segments: a head is followed by a thorax and an abdomen. At first glance, a big difference between the nervous systems of vertebrates and invertebrates may be the position of the nervous system: a dorsal brain and dorsal spinal cord in vertebrates contrast with a dorsal brain and a ventral nerve cord in invertebrates. However, the discovery of developmental genes and their similarities (“homologies”) between, man, zebra fish, mouse and fruit fly, to name only a few of the most studied organisms, has shown that developmental processes mediated by these genes follow similar principles.

Neurobiologists, therefore, had a new look onto brains and nervous systems, a look that confirmed that the nervous systems of all bilaterian animals, including humans and invertebrates, share many common features and may be related. Based on this essential fact, we argue that research on invertebrate animals, which are in most cases more accessible for studies at the neurobiological and molecular level than vertebrate animals, can yield substantial advances and benefits for our understanding of the nervous systems, its architecture and functioning. We maintain, furthermore, that this research can contribute important information for characterizing pathological dysfunctions of the vertebrate nervous system and for conceiving efficient strategies to fight against recurrent diseases. (See **Figure 1** and Footnote 1).

Below, we provide some examples illustrating our statements and underlining why research on invertebrate neurobiology may be essential for Humans themselves beyond the acquisition of pure biological knowledge.

1) Cephalopods like squids and octopods played an essential role for understanding how the nervous system functions.

The basic ionic mechanisms accounting for the electrical excitability of neurons were precisely discovered and studied for the first time in these animals. Neurons generate impulses called action potentials that travel all along nervous circuits to convey specific messages; these signals act as communication signals in the nervous system so that understanding how they are generated allows understanding how the nervous system works. The fact that this discovery was first made in the giant axon of squids, and then extended to vertebrates, underlines the similarity between the invertebrate and vertebrate neural mechanisms. The flow of ions in these nervous systems corresponds to the flow of currents, and Ohm's law of current flow is fully applicable. Due to the characteristic semipermeable properties of the cellular membrane of neurons with their ion channels, an action potential is generated by a very short (approx. 1-2 milliseconds) inward current carried by two positive ions (either sodium or calcium), followed by closure of the respective ion channels and return to the so-called resting potential. Individual neurons, depending on the ion channels they possess, can fire from 10 to 800 action potentials per second, and fire in bursts, beats, or in other more complex rhythms. This historic example shows how research on cephalopod neurons allowed understanding how neurons, including ours, function.

Cephalopods continue to be attractive animals for studying how the nervous system organizes behaviour. Research on the extraordinary motor abilities of cephalopods, which allow them to adopt various body shapes or move and grasp objects with incredible flexibility using their arms, has led to the development of prototypes and "soft robotics" simulating these capacities. In addition, studying their fast communication system, which generates rapidly moving patterns over their whole body, or which produces the most exquisite camouflage patterns, may yield results of general interest for applications in many human (industrial) domains. The discovery of their impressive cognitive abilities, which includes not only learning but also insight, and social learning by observation, has also received wide attention from scholars interested in understanding how cognitive processes may arise in nervous systems that are distant from that of humans (see **Figure 2** and Footnote 2)

2) The existence of electrical synapses or gap junctions was first discovered in the crayfish.

Neurons communicate with each other and with all other target cells by specific contact sites called synapses. These synapses are of a chemical nature and upon the arrival of a nerve impulse (action potential; see above and **Figure 2**), a chemical transmitter is released in portions, binds to the target neuron or tissue where it causes either excitation or inhibition. The delay between the release of the transmitter and the first noticeable response in the target cell is small and can range between 1 and several milliseconds. Therefore, each synaptic contact in a pathway adds to the time required for conduction and from a neurobiological point of view "slows" information transfer. Yet, some behaviours need to be very fast, for example escaping a predator. The crayfish nervous system was chosen to study the powerful escape behaviours of these animals, which respond to potentially dangerous situations by producing a tail flip that either leads to very fast backward swimming if the predator is coming from the front, or first to a somersault and then to a fast backward swimming if the predator comes from behind. It was found that the very fast reaction time between first sensory detection of the predator and the reaction of the crayfish could hardly be explained by the involvement of several chemical synapses. Additionally to the presence of fast conducting, large neurons, contacts made by electrical synapses, or gap junctions, were found, which conduct escape signals immediately, as it is required during flight responses. It was later discovered that such gap junctions (or electrical synapses) are widespread in the central nervous system including our brain and that they play a very important role during development as they do not only conduct electrical impulses without any delay but also allow the exchange of all kinds of molecules between cells (see **Figure 3** and Footnote 3).

3) GABA (gamma aminobutyric acid), a fundamental and widespread inhibitory neurotransmitter, was first discovered in the crayfish.

Inhibitory cells, pathways and synapses are commonly found in all nervous systems. They are extremely important for the functional organization of the brain as neurons need not only to get excited, but also to be inhibited to enhance signal to noise ratio or to suppress reactivity to irrelevant stimulations. γ-aminobutyric acid (GABA) is the neurotransmitter that is responsible for inhibition at the great majority of inhibitory synapses. This discovery was made thanks to research performed in Crustaceans. Work on their neuromuscular preparations showed that GABA was highly concentrated in inhibitory axons dissected from crustacean nerve bundles. This finding was followed by the demonstration that inhibitory neurons in the central nervous system of the lobster also contained high levels of GABA. Furthermore, a specific sodium-dependent GABA uptake mechanism was identified in crustacean neuro-muscular preparations that could serve to inactivate released GABA and stop inhibition.

The function of GABA as an inhibitory neurotransmitter is now well known in the vertebrate nervous system and in that of many other invertebrate animals, where it follows similar principles and mediates similar functions. This example shows how outstanding contributions to the understanding of important questions on our own nervous system such as the principle of inhibitory neurotransmission are being made by using invertebrates rather than primates and rodents (see **Figure 4** and Footnote 4).

4) Basic mechanisms of motor control are surprisingly similar in vertebrates and invertebrates

A fundamental characteristic of animals is that they can actively move, and for doing that, most animals use contractile tissues such as muscles. For example, insects possess striated muscle with the same molecular mechanism of contraction as that of vertebrates. Thanks to such contractions, appendages like limbs or fins or wings are moved. Muscles contract by the action of motor neurons; usually these contractions are rhythmical such as in walking, where a leg alternates between stance and swing phases, or in flying where a wing alternates between up- and down-strokes. Alternating rhythmical activity is generated within the central nervous system, for example, in the spinal cord of vertebrates or the ventral cord of invertebrates. Circuits or networks of neurons called central-pattern generators by neuroscientists, are responsible for this rhythmicity. Central pattern generators function in a completely isolated nervous system without any sensory feedback; they pre-structure the activity which is required for any locomotion behaviour. However, for the correct execution of limb movements during walking, numerous sensory feedbacks are required, not only from external sense organs (exteroceptors) to avoid stumbling or any disturbance of the movement trajectory, but also from internal sense organs (proprioceptors) which monitor tensions, strain or any load forces acting on joints. In general, very similar physical parameters have to be measured during movements regardless of whether an insect or a cat is walking. The more uneven and rough the terrain is, the more important sensory feedback becomes. How this wealth of sensory information is processed within the central nervous system is only partly understood and, in principle, very similar mechanisms occur in all animals. Therefore, knowledge from how invertebrates move are of immediate importance for understanding how we move. One of the most advanced genetic experimental organism is the fruit fly *Drosophila*, an insect for which transgenic fly lines have been developed. This allowed silencing or activating particular neurons in mutant flies, a strategy that is of great importance for understanding, which parts of the central nervous system are involved in motor control. Again, the wealth of information gathered in invertebrate and vertebrate animals leads to a much deeper understanding of particular mechanisms including severe human pathologies of motor systems (see **Figure 5** and Footnote 5).

5) A large marine snail, the Californian sea hare (*Aplysia*), was pivotal for understanding mechanisms of various forms of learning.

Learning and forming a memory are capacities that can be found not only in humans but basically in all animals. Learning is a prerequisite for survival, in particular, as it allows making predictions about an environment that animals need to master to avoid enemies or noxious stimuli, or to find food , partners, and other valuable resources. Just like us, animals have to learn to avoid aversive stimuli, which could be harmful and life threatening, and to find appetitive stimuli, which may act as rewards such as food, a partner or the way back to the nest. But what happens in the brain and nervous system of a learning individual? It was the Nobel Prize winner Eric Kandel who discovered the great value of using an invertebrate animal, a mollusk, with very big neurons to answer this question and unravel the cellular mechanisms that underlie learning and memory formation. The large marine sea hare, *Aplysia*, a mollusk with big-sized and limited number of neurons in the central nervous system (ca. 10 000), offered an opportunity to study the changes in particular neurons after the animal had learned to habituate (i.e. to diminish its response as a consequence of experience) to a repeated tactile stimulus with no life-threatening consequences. Aplysia possesses a mantle which protects a gill and which is contracted by the animal as a defensive mechanism to protect the gill (Figure 6A). This defensive response can habituate upon repeated and regular stimulations of the mantle (or the siphon, a soft, water-expelling structure) with a weak tactile stimulus (a mechanic contact without life-threatening consequences). The mechanism by which this habituation is achieved takes places at the synaptic level, i.e. at the contact place between two neurons. Upon every tactile stimulation, the sensory neurons decrease the release of chemical transmitters onto motor neurons and interneurons so that excitation becomes progressively weaker and no response is elicited anymore. This form of learning is considered very simple and is termed non-associative as the animal learns to respond appropriately to a unique stimulus, the tactile stimulation. Another form of non-associative learning also studied in *Aplysia* is sensitization, the opposite of habituation. In this case, a repeated noxious stimulus such an electric shock at the level of the tail leads to an increase of responses to a tactile stimulation of the mantle or siphon. Again, this could be explained at the synaptic level (Fig. 6B).

In addition, associative learning could also be dissected in the sea hare. In this case, animals learn to establish predictive links between two or more events in their environment. A typical case is Pavlovian learning in which the association between a conditioned stimulus and an unconditioned stimulus is learned. In Pavlov's experiments, the former could be a bell that anticipated the arrival of the latter, the food that the dog was expecting. Dogs thus learned to salivate to the bell sound, which anticipated food delivery. In *Aplysia*, a tactile stimulation to the mantle/siphon was followed by and electric shock to the tail. As the tactile stimulus anticipated the shock, mantle contractions became progressively more important (Fig. 6C). The molecular mechanisms underlying this associative learning were also unraveled in *Aplysia,* and highlighted the role of adenylyl cyclase as a coincidence detector of the signals triggered by the tactile stimulus (calcium influx into the neuron) and by the shock (influx of serotonin, also termed 5-Hydroxytrypthophane or 5-HT) and the concomitant conversion of ATP into cAMP, leading to protein synthesis upon repeated coincidence detection. Similar cascades have been found in several animals, from invertebrates to vertebrates.

Sea hares, like humans, form memories, which result from what they learned. *Aplysia* memory, like human memory, has different phases of different stability and durability. Like in vertebrates, memory can be classified according to these properties into a short term and long-term memory and their molecular underpinnings can be studied, leading to the discovery of basic principles such as the requirement of protein synthesis to stabilize long-term memories. In vertebrates and humans, it was later shown that the basic cellular and molecular mechanisms of learning and memory formation largely correspond to those available in mollusks and insects, and can thus be regarded as evolutionary conserved mechanisms. The fact that invertebrates have a much smaller number of neurons, which are more accessible to network and modeling studies, shows the advantage of using these animals for uncovering the cellular and molecular underpinnings of learning and memory. In addition, a genetically advanced experimental organism like the fruit fly allows studying these phenomena at the level of genes and gene cascades and thus allows characterizing their role and importance for learning and memory (see **Figure 6** and Footnote 6).

6) Aggression as an exemplary study case

Animals and humans alike are required to make decisions in agonistic situations such as when is it best to flee rather than engage in a fight. Aggression is a key component of behavior as it allows the defending or conquering important resources such as mates, food and/or territory. Not surprisingly, aggression is widespread across species, from salmons to chimpanzees and humans. Yet, it can also be found in insects where, for instance, ant colonies may execute lethal raids and fight against each other for the possession of food resources. Precisely, the neural underpinnings of aggression have been unraveled in the case of crickets, and have proved to be common to a large spectrum of species. Male crickets engage in fights over a scarce resource, for example a burrows or a female. These fights occur in bouts of increasing severity and usually end with a winner and a loser. These roles have been associated with the presence and action of certain neuromodulators, i.e. substances that modulate synaptic transmission between neurons and their electrical and biochemical properties. Research on crickets has identified biogenic amines as well as Nitric oxide (NO) as key components of this neuromodulation in an agonistic context. One of these amines termed octopamine (OA), the invertebrate homologue of the vertebrate Noradrenaline, increases the motivation to fight, and underlies stress and energy demanding behaviors. Winning seems to increase aggression and OA levels, but the decision when to flee is independent from OA levels. In this case, the crucial substance is NO, a gaseous modulator that neurons may also release, which dampens aggression and lowers the threshold for when to flee. Crickets can also recover from loser effects and the important substance here is the biogenic amine Dopamine (DA). There is also a role for serotonin (5-HT; see above), which is released specifically after defeat to maintain a low threshold to flee, which is typical for crickets that lose fights. All these results exhibit remarkable similarities to what is known from aggression in rodents. Recent results even indicated that carefully monitoring of male cricket behavior allows predicting its chances in a fight. “Aggressive individuality”, a term common in the analysis of human social interactions, can thus be biologically assessed in the case of an insect. The advantages to study aggression in crickets or fruit flies are manifold because their simple nervous system offers the opportunity to study in detail cellular and synaptic mechanisms underlying aggressive encounters with much higher precision than in vertebrates. Moreover, in the case of fruit flies, which also fight for resources, the whole toolbox of genetic manipulations is also available for such studies, thus opening fascinating perspectives for the characterization of the genetic bases of aggression (see Figure 7 and Footnote 7).

7) Communication via pheromones: a widespread mechanism for animal communication

Pheromones are one of the most widespread and ancestral methods used by animals for intraspecific communication. They are chemical substances that are released to the environment by exogenous glands (glands that communicate with the environment through orifices) and that convey specific messages such as sexual attraction, territorial defense, presence of food, nest entrances, among many others. Given their role in animal communication, pheromones are termed ‘chemical messengers’ as their role is to modify the behavior of receivers in an adaptive manner, i.e. in accordance with the message received. In the absence of language, they play an essential role in animal communication and in the regulation of social interactions between members of a species. The presence of pheromones in humans has been the subject of vivid debates and no clear evidence exists for the existence of this chemical communication in our species. Yet, it clearly exists in a broad spectrum of species from invertebrates to vertebrates, in which hundreds of molecules have been characterized for their role in communication. This research is of fundamental importance, for instance, in an economic context: it has allowed conceiving lures and traps as a biological controlling method against pests and parasites that may affect in a harmful way crops and human health. Interestingly, the existence of pheromones was first discovered in a moth in the context of sexual attraction. Female silk moths release a sexual pheromone made of two components, an alcohol and an aldehyde, which attracts males from long distances given the extreme sensitivity of olfactory receptors on the male antenna to detect these molecules, in particular the alcohol component, which is a long-distance attractant (Figure 8 A-C). The two molecules building the sex pheromone of the silk moth were the first pheromone components to be characterized and gave origin to the term pheromone. Beyond discovering the existence of chemical attractants, this research had important consequences for our understanding of olfactory processes. It characterized the nature and functioning of olfactory receptors and the pathways, which convey and reshape the olfactory messages from the olfactory receptors located in specialized hairs on the antenna to the brain (Figure 8 D,E). Similar architectures exist in many olfactory systems so that the research on pheromones, started with the humble silk moth, has expanded in a dramatic way our understanding of the neural bases of olfaction, besides its fundamental economic importance (Figure 8 and Footnote 8).

8) Understanding pathological dysfunction of the nervous system: fruit flies as a model for Parkinson disease studies.

Parkinson's disease (PD) is a progressive nervous system disorder that affects a considerable proportion of persons in our societies. Symptoms start with a light tremors but the disorder may evolve to produce stiffness, slowing of movement and speech problems, rendering affected individuals unable to conduct a normal life. Together with Alzheimer disease, it constitutes one of the major neural dysfunctions associated to neural degeneration problems. The discovery of a genetic, heritable basis of Parkinson's disease and the identification of genes that are recurrently associated with the emergence of the disease constituted a critical advance for our understanding of the disease. This allowed developing genetic models for the study of the mechanisms of the disease as an important strategy to fight against it. Fruit flies, with their extended genetic tool kit have played an important role in this research. It was possible to create transgenic individuals with tissue or neuron specific expression of dominant mutations, and thus study PD in flies. These mutants do indeed show some of the symptoms that human patients develop. For instance, they exhibit reduced locomotion, loss of dopaminergic neurons, problems with reactive oxygen species, mitochondrial dysfunction, and protein aggregation; these are all symptoms that humans with PD also exhibit.

Studies on fruit flies allowed identifying unknown aspects and genetic interactions underlying PD. For example, perturbation of mitophagy, the selective elimination of dysfunctional mitochondria, is associated with the development of PD. Studies in Drosophila showed that two PD genes, Parkin and Pink1, interact at the level of the mitochondria and play a crucial role in the dysregulation of mitophagy at the onset of PD (Figure 9). Other genes and genetic interactions characteristic of the disease have been since then identified in PD flies, thus enhancing our understanding of the disease affecting humans. Fly neurophysiology thus offers a unique opportunity to progress in the search of medical solutions for PD (Figure 9).

Conclusion

Invertebrates have played a pivotal role for understanding functional principles of the vertebrate nervous system, including that of Humans. Thanks to their large and accessible neurons, which allow recurrent identification and characterization from one individual to the next, invertebrates have pioneered the discovery of basic mechanisms of neurotransmission and neuromodulation. They have been, therefore, fundamental models to increase our basic knowledge about how the nervous system works and is organized. Although this role remains and promises more discoveries for the future, the advent of the genomic era has added a new perspective in the case of invertebrate models regularly used in neuroscience studies. Many of them had their genome entirely sequenced (Aplysia, fruit flies, bees, ants, silk moths among many others), thus opening fascinating perspectives in terms of studies identifying the role of specific genes and their interactions for specific behaviors and pathologies. Identifying the molecular key players of behavioral components, as well as of neural diseases, will be possible using transgenics and mutants as available in fruit flies and other invertebrates. Thus, paying attention to invertebrates and appreciating the potential of their reduced nervous systems constitutes a strategic relevant decision for understanding the human nervous system. As Nobel Prize winner Santiago Ramon y Cajal used to say when comparing the nervous system of insects with that of vertebrates: “*As usually, the genius of life shines more in the construction of smaller than larger master pieces”.*

Figures and Footnotes of this position paper can be obtained from M. Giurfa (martin.giurfa@univ-tlse3.fr) or Hans-Joachim Pflüger (pflueger@neurobiologie.fu-berlin.de)