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Crayfish Brains and the Comparative Approach to the Nervous System*

Die Erforschung der Sinnesorgane, Neurone, Gehirne und des Verhaltens sehr verschiedener Vertebraten und Nicht-Vertebraten liefert eine Vielzahl organisatorischer und funktioneller Parallelen. Diese Parallelen, die man bei Tierarten mit gänzlich unterschiedlicher Evolutionsgeschichte vorfindet, könnten auf bestimmte Selektionsmechanismen zurückzuführen sein, denen die einzelnen Tierarten durch die gemeinsame Umgebung ausgesetzt waren. Man könnte den Sachverhalt auch metaphorisch so umschreiben, daß die Organismen in ihrer Evolution mit »Problemen« konfrontiert waren, für die sie »Lösungen« finden mußten, um sich in einer bestimmten Nische einzurichten oder vorhandene Quellen ausschöpfen zu können. Im folgenden Aufsatz werden einige Beispiele für Form und Funktion von Nervensystemen im Hinblick auf diese Zusammenhänge erläutert.

Summary

The study of the sensory organs, neurons, brains and behaviour of a wide range of vertebrate and invertebrate animals provides examples of many organizational and functional parallels. Such parallels that occur in different animal types with separate evolutionary histories may be the result of the particular selection pressures exerted on different animals by a common environment. Metaphorically speaking one can think in terms of evolving organisms being presented with »problems« and finding »solutions« that allowed them to colonise a particular niche or exploit some resource. Examples of the form and function of a number of neural systems are provided here in the context of this idea.

Introduction

The comparative approach to animal nervous systems is often understood to mean the study of the nervous systems of the vertebrate animals with the ultimate aim of understanding the workings of our own minds. Implicit in this approach is the assumption that the nervous systems of

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the vertebrate animals with their common evolutionary roots are all very similar in design. To a zoologist however, the comparative approach embraces the nervous systems of all animals, including the invertebrates. The goal of the investigation takes on a different form in that the interest is not so much in how our own minds work but in the fundamental principles of nerve cell function. This is a vastly complex matter and has been studied at many different levels using a wide range of techniques. The approach I discuss here takes as its starting point the assumption that the vertebrate and invertebrate animals diverged very early in their evolutionary histories, and that the parallels we find in a comparison of their nervous systems are the result of a specific »need« to overcome »problems« and that the »solutions« to these problems were sometimes so limited that both groups of animals came up with the same »strategies«. The liberal use here of such anthropomorphic terms (problems, solutions, answers, strategies) in no way implies that these processes are at work. The actual mechanisms involved in the evolutionary history of the animals and ourselves are well described but constitute a study in themselves. The metaphors are intended only as a shorthand way of referring to these evolutionary processes, but are useful in getting across a general concept - that of very different animals evolving separately in a common environment.

Let us begin with the idea that the animals we find on this earth developed over a long period of time from single-celled organisms, and that the larger multicellular forms appeared later. Let us also accept for the moment that the evolution of the more specialised forms enabled them to move into previously unoccupied areas where they could radiate and again diversify. We can imagine evolution as an experimental process in which developing life forms continually faced barriers, and the reward for finding a solution to the problem was to survive or even dominate. The conquest of the land by the aquatic animals, for example, depended on finding a way to avoid drying out, to having gas exchange organs that did not collapse (as gills do when they are not supported by water), and to overcoming all the problems of reproduction. These are common problems for all animals no matter whether they are crabs or frogs. When we look at the »answers« that these animals have found, we find again and again that they are astoundingly similar in principle: Gills are replaced by internal lungs that are kept moist in both the frogs and the land crabs; intromittent organs transfer the sperm from male to female, and the embryos are protected in eggs with shells that resist dessication.

Receptor Organs

Let us close in on this idea a little, and see how it applies to sense organs. Sense organs are the windows through which we experience the world. They have the property of converting, (transducing) various kinds of physical energy, be it light, heat, pressure, sound or whatever, into signals in the nervous system. Our sensory windows are relatively narrow. We cannot hear very high pitched sounds, we cannot see beyond deep red or blue, we cannot smell chemical substances at very low dilutions. So there is a great deal going on that we don't know about, and are inclined not to believe, unless, as in the case of radio waves, we can artificially change (transduce) the energy to that which falls within one of our windows.

Sense organs have been developed to serve different purposes, and can be thought of as filters. Photoreceptors respond to light, phonoreceptors to sound and so on. Important for our concept though is that all animals are subjected to the same set of physical parameters. We all have to cope with the same light levels, the same force of gravity and the same density of water or air. Thus developing a receptor for sound carried through the air is a problem common to men and crickets. Is there a »best« answer to the problem?

Consider the receptors for light. Suppose the first animals had no receptors for light. One can imagine that the possession of a receptor for light would benefit a small motile (animal) cell that perhaps obtained its nourishment by eating other small non-motile (plant) cells. The non-motile cells must have light to grow, and so flourish where it is light. Thus if the motile cell could find its way to light it would increase its chances of finding food. One way to transduce light energy into a form that can be perceived by the animal is to have a particular chemical, a photopigment, which when struck by light changes its form and releases energy. That this is a good method (the only way?) for the animals can perhaps be deduced from finding that they all do it this way. The actual photopigments are different, but the principle is the same and is already present in the very earliest forms.

Equipped with such a photoreceptor an animal can detect not only the presence or absence of light, but whether it is decreasing or increasing. With one single photoreceptor though, it cannot detect whether an object near it is moving, nor in which direction, nor how fast. Here is a nice problem. We can imagine advantages in being able to see the direction and movement of objects in the environment around us. We know that the highly developed animals can do this. Mice can see us coming and so can cockroaches, and what is more their evasive actions leave us in no doubt that they can estimate the direction and velocity of our movements quickly and accurately. But their eyes appear to be very different - the mouse eye has a single large lens, the cockroach a large number of small lenses fused into a compound structure. But are they different in principle? The problem is to design a receptor that will detect light and also detect the direction and velocity of movement of a contrasting light/dark boundary. One way is to build an array of individual receptors which in themselves can only signal the change in the light intensity they receive. However by comparing the outputs from the neighbouring individual receptors as a light/dark border passes over the eye, one can deduce the direction and velocity of movement of the border. If all the receptors point in the same direction, the eye would possess a high acuity but have a very narrow visual field. Arranging them in a circle provides a greater field of vie although one must trade this off against the loss of acuity. Looking again at the eyes of the mouse and the cockroach, the large single lens of the mouse eye directs an image of the objects in its field of view onto the concave array of receptors that go to make up its retina. Any movement of a light/dark boundary in the visual field will therefore result in a movement of a light/dark edge across the receptors. The cockroach has a compound eye made up of a large number of very small single lens eyes and these are arranged convexly in a semicircle. Movement of a light/dark boundary in its visual field will also result in a sequential change of the light intensity in the receptors of the eye. In both eyes the result is exactly the same. In principle there is apparently only one good way to build an eye to detect the direction and velocity of an object moving in its visual field. The choice of the large single lens eye or the multifaceted compound eye is influenced by some nice optical problems relating to the light gathering and focussing properties of optical devices, and the detailed needs of the separate animals.

Eyes are only one example of the remarkable convergence of separate evolutionary histories. Parallels can be found in the receptors for balance, sound, gravity and olfaction. At this level, little separates the invertebrates from the vertebrates.

Neural Organization

Behind all sense organs in all animals lies a nerve, and the sense organs transduce the physical energy into signals in the nerve. These electrical signals are short pulses, and there is a universal code used by the nerves of *all* animals. The greater the intensity of the stimulus, the greater the frequency of the nerve impulses. It is a simple matter to record these signals from the sensory nerve, stimulate the sense organ and observe the increase in frequency when the intensity of stimulus is increased.

The choice of this common system of information transfer has farreaching effects on the neural organisation because the information in the sensory nerves is carried to the brain. The significant point is that the sense organs have transduced the various energies of the world into trains of pulses in which only the intensity of the stimulus is coded. There is nothing in the signal itself to say whether the signal which produced it was light, heat, noise or whatever. This is lost. The qualitative nature of the stimulus is preserved only in that it is contained in a particular line. The central nervous system »knows« that a light has gone on or off, only because that particular labelled line is activated. In such a system it is imperative that the lines are accurately addressed to the appropriate areas of the brain, and that there are such areas. The moment such a signalling system is chosen, where the qualitative nature of the stimulus is preserved only in a labelled line, certain constraints are set on the design of the central nervous system, and if the same system is adopted in both the invertebrates and the vertebrates, and this is the case, the same constraints must apply.

Gradually we are led to the following idea: The sensory systems of animals all operate along the same lines, and evolution seems often to have chosen the same way to do something several times. The movements of animals are all effected by contractile tissue, muscle, and the same molecular mechanisms, are used to get tissue to contract. No muscles are able to actively elongate, placing certain constraints on how animals' bodies are designed. Between receiving a stimulus and issuing a command lies a collection of neurons, and destruction of this centre produces severe disturbances in the behaviour of animals.

The hypothesis that follows is that the principles used by the central collection of neurons to sort out incoming information, combine it with past experience and issue a motor command are the same in all animals. To test the validity of this hypothesis it is worth looking at the brains of the invertebrates, firstly to see if our suspicion of similar neural mechanisms holds, and further to see if we can understand the neural bases of behaviour in a simpler form.

Brains

It has been known for a long time that there are areas in the human brain that receive particular signals and are responsible for particular functions. These areas can be anatomically identified in brains that have been preserved, removed from the skull and sectioned in different planes. It is possible to follow the incoming nerves that go to the various »nuclei« or collections of central neurons.

What then is the situation in the invertebrates? Where does the crayfish have its brain? Does it have any recognisable divisions associated with sensory inputs?

The crayfish brain is about 2.5 mm across and is situated between its eyes. It can also be preserved and removed from the animal and sectioned in various planes. Because it is much smaller than the brain of a man, it is possible to cut it into very thin sections, reduce the entire brain to an ordered series of slices and mount these on a microscope slide. The sections are treated with stains so that the neurons can be seen with the microscope, and their distribution within the brain reconstructed.

From such sections, areas of the crayfish brain can be determined that receive specific inputs from sensory organs. Furthermore it can be established that the crayfish brain is bilaterally symmetrical like that of most other animals, and that all the brains of a particular species look exactly the same.

1. Synapses. A closer look at the neural tissue reveals a tangle of axons and dendrites in which no orthogonal geometric order can be determined that would allow a prediction of the connectivity between the individual elements. Using the electron microscope to look even closer does not reassure us: The smallest branches are less than 0.5 thousandths of a millimeter. What is visible though are specialised contacts between neurons. These junctions are now recognised to be the regions where the information passes from one cell to the next, and are called synapses. There is good reason to believe that the presence of vesicles in one of the two cells marks it to be the presynaptic cell, and hence allows the direction of the information transfer to be determined. It is difficult to tell at the level of the electron microscope whether one is looking at invertebrate or vertebrate tissue. Both use the same method of transferring the information between the neurons: A chemical, called a transmitter substance, is released by the presynaptic cell when its membrane potential changes. The released transmitter crosses the synapse to the postsynaptic cell, binds with specific sites on the postsynaptic membrane, and produces a change in its permeability to certain ions. This leads to a change in the membrane potential of the postsynaptic cell.

2. Establishing connectivity. Given the difficulty of determining the exact connectivity between neurons by anatomical means, pathways have to be followed by making electrical recordings. Electrodes can be placed within individual central neurons without unduly damaging them and a stimulus applied to a sensory organ. There are, however, some problems. The neurons are small and we have to expose the brain to place our electrodes. In the vertebrates electrodes can be mounted on the skull and lowered through a small hole into the brain. But the brain of a cat, or even a mouse is huge, containing about 10 to the power 10 individual neurons. The crayfish brain on the other hand has five orders of magnitude fewer

neurons and with perfusion will continue to function even when it is isolated from the body of the animal. Invertebrate neurons are no smaller than those of the vertebrates, in fact in many instances they are slightly larger.

Identified Neurons.

What can one find out about crayfish and other invertebrate brains from such recordings? Are their sensory inputs arranged in specific ways? Yes they are. Are the areas of the brain always organised in the same way so that the line labelled system will work? Yes they are. At this level the invertebrates are the same as the vertebrates. Is that all that has been discovered? No. The most exciting finding is that many of the neurons in the invertebrate brains are individually recognisable and are found again and again in the same place. Individual motorneurons can be recognised not only by their shapes and position in the brain but by their function. They always receive the same sensory inputs and always innervate the same muscles. Unlike the vertebrate, invertebrate muscles are often innervated by no more than five motorneurons and there are now many cases where all the motorneurons of a particular muscle have been individually identified.

The enormous significance of this discovery can be appreciated by considering the following analogy: Suppose we were provided with the task of finding out how a small pocket calculator worked. We have an unlimited supply of the calculators to dismantle, but we are never able to reassemble any part which has been dismantled. We would proceed by carefully taking the devices to pieces, and noting the position of the various components, perhaps even measuring the electrical currents that flowed in the components while the calculator was operating. If we were to find that each calculator was unique we would soon despair. The discovery though that all had the same circuit, and that all the components were in the same place and many individually identifiable as to their shape and function, would mean that we could progress. We could assume that what was true for one was true for them all. The same can be assumed to be true, to a certain level, in the brains of the invertebrates. Some aspects of the behaviour we see in the intact animal can be understood in terms of the logical interaction between neurons in the brain that are arranged in a describable way.

This is only the beginning. Simple reflexes in both vertebrates and invertebrates have been known for a long time to be the result of simple circuits. As attempts are made to explore more complex behaviour patterns, where more neurons are involved, so the interpretation becomes more difficult. Simultaneous recordings from sets of selected neurons would reveal important information about the lateral interactions between the members of such sets but this is technically very difficult to achieve. Simple concepts concerning the transfer of information from one neuron to the next using a chemical transmitter have had to be revised following the discovery of a whole host of chemical substances that can have subthreshold, or modulatory effects. It is suspected that neurons can change the molecular make up of parts of their membranes. Changes of this nature occur in the central neurons of sea hares and are correlated with learning, and it is highly probable that a similar process occurs in the vertebrates where learning can be prevented by the injection of protein synthesis inhibitors.

Uniquely Optimal Solutions?

The extrapolation from one system to another, or from one animal to another must be done with caution. There are many examples of where a common solution, in principle, has been found for a common problem but where the details of the solution differ. On closer examination these differences may be seen not to reflect better or worse ways of solving the problem, but the best solution for that particular animal. Thus although men and swimming crabs both exploit the physical properties of fluid filled canals to detect angular accelerations of their bodies and maintain their balance, man has three canals, the crab only two. A careful look at the role played by these sense organs in the behaviour of the two organisms, shows that the crab can solve its particular balance problem perfectly with two canals, and three are not necessary. Similar differences in the details of the solutions to common problems can be found at all levels. The basic mechanism underlying learning and memory in all animals may turn out to be the same at the molecular level, but the range in the complexity of what is learnt will certainly be very different, and behaviourally specific. Bees have colour vision and the neural machinery to associate colour and a food source. Crayfish are colourblind - there is no point in their possessing the bee's facility, instead there is some evidence to suggest that they can learn to distinguish between water-borne vibrations of different frequencies.

The comparative study of nervous systems has been responsible for the identification of a number of basic principles of nerve function and is therefore an acceptable approach. The idea though, that the solutions to the evolutionary problems are the same in different animals because they are uniquely optimal has been questioned. A counter-proposal favoured by some developmental biologists is that organisms have a limited number of strategies available to them, that these were all present at a very early stage and that they are employed as the need arises. Thus the similarities between the solutions are not so much due to the common problems, but to the limited means by which they must be solved. Whatever the correct view, one aspect is unchanged: the basic principles governing nerve function in a wide range of animals are the same and can be revealed by the study of any animal.