ADVANCES IN SENSORS; THE LESSONS FROM NEUROSCIENCES

M. Costa
Department of Physiology and Centre of Neuroscience
School of Medicine
Flinders University
Adelaide 5006
Australia

Abstract This is a short review of how neuronal sensors fit in the broader biological context of animal survival. This may help those involved in the development of engineered sensors to put in perspective their task with what the evolutionary process has achieved. Most of the information reported here is available in the educational field of neuroscience, with mention of some recent relevant findings. I have attempted to place these findings in an evolutionary perspective as it clarifies better the intrinsic role of some of the extraordinary particularities of the biological solutions of neuronal sensors.

Keywords:
action potential; axon; ion channel; mechanoreceptor; membrane potential; neurons; neurotransmitter; olfaction; proteins; synaptic gap.

1. Energies that affect earth living organisms survival

Organisms are exposed on earth to an environment with different physical energies, including gravity, and in general kinetic energy, chemicals, temperature variations, sound and noise, and electromagnetic waves. Evolutionary processes, not too surprisingly, have endowed living multicellular organisms with sensors specific for these physical energies within some selective ranges, mainly those best represented on the earth's surface. Such sensors are intrinsically linked with the ability of organisms to behave in an appropriate way, 'taking in account' the sensed environment. This implies the ability to comply with essential functions of all living systems. These include escape from harm, feeding, breathing and drinking, reproducing and exploring. Escape from harm is the fun-

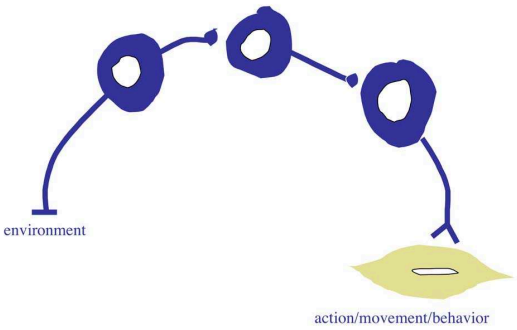

Figure 1. Rudimentary nervous system

damental capacity that ensures the survival of each individual organism and leads, in humans and most vertebrates to the simple withdrawal from painful stimuli. Feeding is required as food is the fuel needed to be burned to provide the energy, which is stored as chemical energy by living beings. Breathing guaranties the intake of oxygen for the oxidation (burning) of the food fuel. Most living chemical reactions occur in water, an essential component of life on earth. Individuals disappear (die) and are replaced by other derived individuals similar, but not identical, and this enables 'species' to change adaptively by the evolutionary process of natural (Darwinian) selection. A great deal of animal behaviour is dedicated to the reproductive function. Finally living organisms show spontaneous motion (exploratory locomotion). This gives the advantage of most animals versus plants, to find suitable new environments rather that adapting to an adverse local one. Human migration in prehistory from Africa testifies to the importance of this function for the success of human kind on earth.

2. The emergence of a nervous system

The most significant occurrence in evolution of multicellular organisms is the differentiation of cells that can initiate and carry signals at large distances within the organism. Such cells are called nerve cells or neurons. The main property of such cells is the ability to sense and amplify very small signals to initiate macroscopic behaviour of the entire organism. The diagram in Figure 1 shows the minimal organization of a nervous system with a sensory neuron that responds to some physical

energy, a motor neuron that acts on a movement cell (muscle cell) and in between an 'inter-neuron'.

As multicellular organisms became more complex in evolution, including humans, the relative number of interneurons increased. Most of the human brain and spinal cord is made of interneurons. Although the diagram is highly simplified it implies that each of the three classes of neurons will have quite different functions. The sensory neurons, also called primary afferent neurons, are the link between the organisms as a whole and the environment (including its own internal environment). The sensory neurons detect stimuli in a very localized fashion and at a particular, albeit ongoing, time. Yet the organism needs to detect changes in the environment over larger areas surrounding the organism and over longer time periods. This involves the detection of what, where and when something is 'out there'. The 'what' requires neural processes of construction of some 'percept' involving many neurons. The 'where' involves construction of neuronal maps of some sensed information (visual, acoustic, body etc. maps). The 'when' involves the inaccurate process of memory and prediction that enable past events to affect behavior and future events to be predicted to some degree. Different time range memory systems are known in higher vertebrates and these include working, short and long-term memory. Since the essential elements of the nervous system are the nerve cells, a short description of the unique properties of neurons follows.

3. Neurons as excitable cells

Neurons are specialised cells with a typical shape characterised by a cell body, in the range of several tens of micrometres, and long cytoplasmic processes, nerve fibres either called axons or dendrites. The identity of nerve cells as being formed by cell body and fibres has been recognised since the first half of the nineteenth century, when a young student, Deiter, dissected a large nerve cell from one of the collections of nerve cells (nuclei) in the brainstem. Towards the end of the nineteenth century Camillo Golgi, an Italian neurologist, developed a stain that highlighted extremely well only a few nerve cells from a brain packed with myriad nerve cells. This staining method enabled Ramon y Cajal, a Spanish histologist, to confirm his theory that the nervous system is made of individual nerve cells (neuron theory). Neurons, as most other cells in multicellular organisms, share the machinery for life, including burning fuel, synthesis of its own components (e.g. proteins) and excretion of residues. In particular neurons have very little store of fuel to burn and thus need a continuous supply of fuel and oxygen. In addi-

tion, neurons and muscle cells share the characteristic of 'excitability'. Excitability is a term that implies responsiveness of a cell to a small signal leading to a disproportionately large response. Neurons are in an electrical state distant from equilibrium. In a typical neuron the charges on the internal cytoplasmic fluid are more numerous than on the external liquid, producing a difference in potential of about -65mv. This means that they have an electrical polarity. The transient sudden loss of such polarity is what has been named action potential, nerve impulse or spike. In order to understand how this loss of polarity occurs we must first understand how the electrical polarity is generated.

Neurons, like all other cells, are surrounded by a cell membrane made of a phospholipid bilayer, which is self-assembled in an aqueous environment. Within this compartmentalised 'bag', the cytoplasm with organelles, performs the specific cell functions. The electrical polarity of the cell is due to the presence in the membrane of a large macromolecule that actively separate the ions Na+ and K+, the sodium-potassium pump. This molecule is a protein. All proteins are formed by single strands of sequences of the basic elements, aminoacids. These are small molecules made in the cells or imported from the outside, characterised by an amino group at one end and by a carboxylic group at the other. These endings make aminoacids capable of binding together in strings (linked by peptidic bonds). Such strings form peptides and proteins. The single strand is usually bent to form spirals, which then form rods and globules. The final shape of a protein thus depends on the 'tertiary' structure. The sodium-potassium pump is a protein with a pore that can open and close inside or outside the neuron membrane, transporting sodium ions out and potassium ions in the neuron. This pump ensures that there is little sodium inside the cell and more potassium than outside. Since there are fixed negative charges anchored in the molecules inside the neuron, this pump maintains the electrical polarity of the nerve cell. Thus this polarity keeps the cell membrane at 'rest' far from its equilibrium. If the ions were able to move freely across pores in the membrane they would rush across along what is called the concentration gradient until the rush is counteracted and balanced by the charges reaching their 'equilibrium potential'. Indeed there are special proteins in the membrane, which form pores, normally closed or almost closed and that are specific for different ions. The main determinants of the neuronal activity are channels for sodium and for potassium, which have a remarkable property of opening in response to a change in voltage off the membrane. When the membrane becomes less negative (it is said then to depolarise), these channels open with a positive feedback process. This non-linearity ensures that past a 'threshold' value of

the membrane potential, the sodium channel opens in an explosive-like manner, enabling sodium ions to rush inside the cell along its concentration gradient until it reaches its equilibrium potential (around 40 mv positive). This process lasts only about one millisecond before stopping spontaneously, and is followed by the opening of the potassium channel, which is also voltage dependent. The potassium ions rush out, removing the excess of positive charges from the inside, thus re-establishing the electrical negative polarity. This transient change in polarity of the neuronal membrane (depolarisation followed by repolarisation) is the event called action potential. Other ions such as chloride and calcium also play minor roles in this process. This explosive, self amplifying, all-or-none event, is the way in which nerve cells carry signals. The entire language of the nervous system is coded by action potentials.

Understanding of the structure and function of these channel molecules has developed enormously in recent years thanks to molecular biology and the technique of patch-clamping, which enables one to detect not just the electrical events in the entire neuron, with a microelectrode inserted in the nerve cell, but the electrical events in small patches of neuronal membranes, sucked by a glass pipette, containing a single channel molecule. The action potential sets currents that affect the neighbouring patch of membrane, which then becomes locally depolarised. This 'passive' depolarisation of adjacent patches of membrane triggers there the generation of a new action potential. The sequential activation of local action potentials results in the 'conduction' of action potential along the entire length of the neuronal membrane. As the neuron has very long processes (axon), this signal travels from its origin to the end. This is the propagation of nerve impulses within the nervous system. The speed of conduction depends on the diameter of the axon, the larger the greater the speed. As greater speeds are advantageous for fast responses, in evolution most axons have become covered by fatty sheets secreted by satellite cells (glial cells), to insulate long stretches of the axons compelling action potentials to jump along the axon, thus increasing its speed of conduction. The speed ranges from a few cm/s up to 120m/s. These electrical signals can also jump from one neuron to a next neuron in the circuit. This is called electrical transmission. This mode of transmitting neuronal impulses is the exception rather than the rule in the nervous system. The most common mode of transmission is via a chemical, which is released at the end of a neuron, acting then on the next neuron. This 'chemical transmission' is mediated by several types of small molecules, thus named neurotransmitters. Amongst these are amines such as acetylcholine, noradrenaline, adrenaline, dopamine and serotonin. Also aminoacids such as glutamate, aspartate, glycine and

GABA are widely used transmitters. Also ATP, the molecule usually associated with chemical energy in cells, and nitric oxide act as transmitters. Several small neuropeptides, short sequences of amino acids, act as transmitters.

The process of chemical transmission involves the arrival of an action potential at the nerve ending. This triggers the entry of calcium ions in the cells via specific protein channels. Calcium triggers an amplifying cascade of chemical reactions between macromolecules and small vescicles (bags) containing several thousands of neurotransmitter molecules. This results in the opening of these vescicles (synaptic vescicles) to the outside of the neuron. When the vescicles open and release the transmitter, the distance to the next neuron is very small (on the order of tens of nanometers). This gap is called synaptic gap and the 'synapse' is the ensemble of the 'pre-synaptic' membrane, the gap and the 'post-synaptic' membrane. Transmission due to the diffusion of the neurotransmitter is very fast, being measured in microseconds. The postsynaptic membrane (i.e. the membrane of the next neuron) contains special proteins that selectively bind the neurotransmitter molecules. These proteins are called 'transmitter receptors' and form an enormous variety of molecular families adapted to the tens of different transmitters as keys to their locks. The binding of transmitters with their receptor triggers an amplifying cascade of events inside the neuron that leads to opening or closing of ion channels, thus changing the electrical state of the membrane. If the transmission leads to a depolarisation, and consequently to the triggering of an action potential, it is said to be excitatory. Transmission can also be inhibitory if the result of transmitter action is the increase in polarity of the membrane (hyperpolarisation) taking the neuron further away from its threshold of firing action potentials. Indeed much of the control of neuronal operation in the nervous system involves inhibitory transmission, without which the nervous system would run wildly uncontrolled. The entire armamentarium of pharmacological drugs used for pleasure, such as morphine and heroin, cannabis, ectasy, psychedelic drugs, most poisons (e.g. sarin), some pesticides and most therapeutic drugs that affect organs (e.g. high blood pressure), human behavior and mental diseases (e.g. schizophrenia, depression, Parkinson), all act on some of the processes involving chemical transmission. The function of nerve cells and the neural circuits they form is thus determined by a number of highly non-linear amplification processes that ensure that very small signals are amplified to become macroscopic events.

4. Sensory neurons

The first neuron in the simplified neural circuit in Figure 1 is the sensory neuron. The sensory neurons share all the properties of other neurons such as excitability and transmission to other neurons via chemical transmitters. They are activated from their resting state by events in the environment. Different classes of sensory neurons then exist to detect the physical energies mentioned above. These are the true 'biological sensors'. Of course all living cells are capable of responding to changes in the environment to some degree and thus are also 'sensors'. The activation of sensory neurons can be direct or may involve the activation of an intermediate, non-neuronal cell (sensory cell). The following list describes the major classes of sensory neuronal receptors according to the physical energy they sense.

Classification of neuronal sensory receptors

- Mechanoreceptors
  - kinetics muscle, joints, pressure on body position and movements of body parts (sense of self; proprioception)
  - internal sensors for tensions and pressures (heart, blood vessels, gut, bladder, lungs etc.)
  - hearing and balance
- Chemoreceptors
  - feeding
    - olfaction (olfactory lobe)
    - taste
    - internal sensors for nutrients
  - body homeostasis (control of sugars, blood O2 and CO2 etc.)
  - defense (tissue damage)
  - reproduction
    - olfaction (pheromones and neuroethology)
- Photoreceptors
  - vision; photons receptors

5. Sensory transduction

The process by which a small change in the environment is sensed and amplified to become neural activity is called sensory transduction. Since different sensory neurons respond to different stimuli (small changes in the environment), neurophysiologists predicted the presence of special molecules on the surface of the nerve endings of the sensory neurons or of the sensory cells, specifically adapted to respond to different stimuli. Each of these molecules should be able to translate the stimulus in a depolarisation of the sensory neuron that would trigger action potentials, thus initiating sensory neural activity. This is achieved by the opening of ion channels by the stimulus, which leads to the graded depolarisation of the sensory ending.

The prototype of sensory neuron studied earlier because of its accessibility, is a mechanosensitive sensory neuron with nerve endings in the skin, surrounded by a small capsule forming an onion-like passive structure, named after its discoverer 'Pacinian corpuscle'. Controlled graded deformation of this corpuscle results in a graded depolarisation of the nerve ending (receptor potential). When the depolarisation reaches a threshold this will trigger action potentials, which will be conducted towards the central nervous system. There is a very good range of stimuli within which the receptor potential and the resulting frequency of firing of action potentials are linearly correlated. The stimulus-action potential frequency response curve is in fact sigmoid, as are most biological transfer functions.

The excellent relation between amount of membrane depolarisation and frequency of firing of action potentials has been demonstrated in most neuron types. Thus frequency of firing of action potentials is probably the most important determinant in coding intensity of sensory signals (stimuli). In addition to frequency of firing different neurons, including sensory neurons, possess additional ion channels that either lead to spontaneous depolarisations or to oscillations that lead to specific windows in which action potentials can occur, resulting in patterns of firing in bursts. The pattern of firing of action potentials is the other major, less well understood, way to encode neural information.

6. Molecules of sensory transduction

The molecular nature of the neuronal receptors is now becoming understood with the advent of molecular biological techniques. The molecular structure of the mechanosensitive channels has been established only recently. In principle mechanosensitive channels must be opened by mechanical deformation of the neural membrane in which they are em-

bedded, either by the stretched membrane pulling on the molecule or via connections with the intracellular 'skeleton' of the neuron itself. One of the simplest models studied is the mechanosensitive channels in bacteria [11]. There is a plethora of families of molecules with mechanosensitivity including: DEG/ENaC family, TRP families, MSC families, some K+channels etc., and these are present in the entire spectrum of living species. One of the recent findings on mechanosenstive channels relates to the hearing system.

7. Hearing system and mechanosensation

The hearing apparatus in terrestrial animals transforms sound waves into nerve impulses. This is performed by a special set of sensory receptor cells (hair cells) located in the organ of Corti in the inner ear. These cells in turn transmit the message to the primary sensory neurons for hearing (an example of indirect activation of sensory neurons). The hair cells are so named because they have a number of small protrusions-like cilia or hair that are bent by the motion imparted by the sound waves that vibrate the eardrum membrane, with amplification of such vibration by an ossicular chain of levers to impart a similar vibration, via an internal liquid, to a thin membrane on which the hair cells are sitting. The bending of the hairs results in depolarisation of the hair cells, which then transmit a chemical signal to the sensory neurons, modifying their neural activity (frequency of firing). The molecular mechanisms by which the hairs, or cilia, open the mechanosensitive channels and their location have been elucidated [3]. The mechanosensitive molecule belongs to the class of the TRP (Transient Receptor Potential) channels previously identified as the gene product defective in a blind Drosophila mutant (see [8]). Molecules with similar structure (homologs) have now been found in many animals, both invertebrate and vertebrate. The TRP superfamily consists of 28 mammalian members as well as 13 Drosophila proteins [4]. These channel protein molecules have a molecular architecture similar to that of voltage-gated ion channels, with four subunits arranged to form a channel. TRPs are generally non-selective channels for positive ions. TRP channels are conspicuously involved in the mechanosensory sensory function. However they are also involved in many other sensory functions including vision, taste, olfaction, pheromone sensitivity, osmosensitivity, nociception and thermosensation. This is surprising and points to a fundamental similarity between molecular mechanisms unrelated to the classic distinction of the sensory 'modalities'.

Some of the TRPs are activated directly by sensory stimuli, but others are activated by a variety of intracellular chemical messengers [12] Initial

studies demonstrate that these sensor molecules can be integrated with microchips for potentially enormous artificial uses [9].

8. Temperature receptors

In mammals, four TRPVs (members of the vanilloid subfamily of TRP channels) are activated at distinct heat thresholds (33–52 ^{o}C), whereas TRPM8 (of the melastatin subfamily) and ANKTM1 are activated at cold (17–25 ^{o}C) temperatures (see [12]). A big surprise in this field is that these molecules were found to also respond also to a variety of well-known natural chemicals from the external environment. These include hot chilli (capsicum sativum, capsaicin) receptor channels, found by Caterina et al [2] to be also sensitive to heath. Other natural substances that activate TRP receptors include cold mint (menthol, eucaliptol, anisette), piperine (black pepper), resiniferatoxin (euphorbia resinifera-cactus), camphor (cinnamonum camphora-laurel), isothiacyanates (mustard, wasabi, horseradish), cinnamaldehyde (cinnamon oil), THC (marijuana; cannabis sativa), allicin (garlic; allium sativum) [12]. Naturally the burning pain caused by capsaicin directly links temperature receptors with pain receptors.

9. Pain receptors

Pain is one of the most mysterious of the senses and certainly one, which although essential for survival, receives little sympathy by the 'users'. Noxious (harmful) stimuli to the skin are known to elicit two kind of painful sensation (see [7]). One occurs earlier (fast pain) and is due to direct activation of pain fibres (depolarisation of axons) by excessive mechanical activation and involves channels of the ENaC family. The second pain is felt with some delay (slow pain) and is initiated by acid (hydrogen ions) acting on the receptor channel TRP-VR1 and the ASICs. The pungent pain produced by capsaicin is also due to its action on the receptor channel TRP-VR1. Noxious heath acts via the TRP-VR1 and the VR1-L. When tissue surrounding the pain nerve ending is injured, a soup of chemicals is produced and released locally. Many of these released substances are able to initiate or positively modulate the activation of pain fibres. Examples of such substances are histamine, serotonin (5HT), ATP, bradykinin, prostaglandins, PARs, neuropeptide such as tachykinins, bradykinin, CGRP etc., and different nerve growth factors. It is still uncertain whether there is also a formation of endogenous capsaicin-like substances (endogenous vanilloids). Conversely there are also substances capable of reducing the excitability of the pain nerve ending. Amongst these are the cannabinoids (endogenous molecules such

as anandamide or exogenous ones like THC, the active principle of marijuana), opioids (endogenous molecules like enkephalins, endorphins and dynorphins, or exogenous ones like heroin and its derivative morphine). Thus peripheral receptors for pain abound and modulating mechanisms render the field most complex for suitable control of this defense function. Chemical sensors par excellence are the olfactory and taste receptors.

10. Olfaction

Olfaction, once thought to be a primitive sense, is now recognized as an elaborate sensory system that deploys a large family of odorant receptors to analyse the chemical environment. Interactions between these receptors and their diverse natural binding molecules (ligands) translate the world of odors into a neural code. Humans have about 350 odorant receptors. Rodents have more than a thousand.

In vertebrates the neurons for olfaction are located in the nose mucosa and consist of short neurons with a peripheral ending endowed with odorant receptors for a large number of molecules in the environment. Each receptor neuron only contains one odorant receptor and is connected directly with the olfactory lobe of the brain. The vertebrate olfactory system must cope with a staggering developmental problem: how to connect millions of olfactory neurons expressing different odorant receptors to appropriate targets in the brain.

The story of search for odorant receptors has come of age in recent years with the awarding of the Nobel price in Medicine in 2004 to two investigators — Dr. Richard Axel and Dr. Linda S. Buck — for their discovery in the early 1990s of the genes that code for odorant receptors in the rat [1]. The discovery of odorant-receptor genes in the rat provides a missing link between the molecular biology odorant receptors and the physiological properties of sensory neurons. Indeed the odorant receptor also determines the connectivity of the sensory neurons, as shown in experiments in which the receptor has been removed from single olfactory neurons and replaced by different ones [5, 6].

All animals exhibit innate behaviors in response to specific sensory stimuli that are likely to result from the activation of developmentally programmed neural circuits. Even the activation of single classes of olfactory neurons can trigger complex behaviors [10]. The authors observed that Drosophila exhibit robust avoidance to odors released by stressed flies. When stressed, the flies emit an odorant mixture that elicits avoidance in other flies. CO2 is the active component of this mixture. Specific blockade of the activation of a particular odorant receptor

also blocked the response in the other flies. Thus CO2 is the signal molecule for this avoidance behavior.

11. Vision

Vision is undoubtedly the most important of the human senses. The process of transduction of photons into neural activity occurs in the retina by a process with significant similarities to chemical receptors including odorant reception. Photons reach the retina via the optical system of the eye and activate special protein receptors (the visual pigments). These are located on the surface of the receptor cells (non-neuronal) called cones (separate classes containing pigments for red, green and blue wavelengths) or rods (with a broad spectrum pigment of wavelength, thus suitable for black and white night vision). Photons acting on the receptor induce a conformation change of the protein and this initiates a cascade of chemical events inside the receptor cell that leads to a change in its electrical property with chemical signals, in turn acting on the primary sensory neurons in the retina.

12. General view of the sensory systems

Despite the large variety of stimuli the sensory neurons respond to, the mechanisms of activation (transduction) utilize remarkably similar molecular processes. However, their activation is only the first step in the process of constructing and utilizing the sensory information. Neurons work in large groups and, as signals go through subsequent neural synaptic stations, undergo modifications all resulting in a simplification and amplification of relevant features. One of the most common mechanisms is lateral inhibition. Activation of parallel sensory pathways also activates cross-talking neurons, which are inhibitory to their neighbor. The result of such an arrangement is that signals with a small advantage in amplitude become larger at the expense of the surrounding weaker signals. By the time the signal reaches the cortex, the original weak but larger signal becomes amplified. This enables a better discrimination of what happens in the outside world. A similar process of lateral inhibition in the retina ensures a better detection of edges for shape recognition. Sensory neural systems may well be a good lesson for those involved in the important task of developing better sensors for a variety of aims. In parallel to developing better amplification and discrimination of signals, it is equally important to improve the quality of analysis of the information for decision-making that should be based on wise knowledge, avoiding the proverbial badly adaptive ‘knee jerk’ reactions.

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