Plant Signaling & Behavior 9, e27886; January; © 2014 Landes Bioscience

Plant anesthesia supports similarities between animals and plants

Claude Bernard's forgotten studies

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The French scientist Claude Bernard (1813–1878) is famous for his discoveries in physiology and for introducing rigorous experimental methods to medicine and biology. One of his major technical innovations was the use of chemicals in order to disrupt normal physiological function to test hypotheses. But less known is his conviction that the physiological functions of all living organisms rely on the same underlying principles. He hypothesized that similarly to animals, plants are also able to sense changes in their environment. He called this ability "sensitivity." In order to test his ideas, he performed anesthesia on plants and the results of these experiments were presented in 1878 in *"Leçonssur les phénomènes de la vie communs aux animaux et aux végétaux."*[1] The phenomena described by Claude Bernard more than a century ago are not fully understood yet. Here, we present a short overview of anesthetic effects in animals and we discuss how anesthesia affects plant movements, seed germination, and photosynthesis. Surprisingly, these phenomena may have ecological relevance, since stressed plants generate anesthetics such as ethylene and ether. Finally, we discuss Claude Bernard's interpretations and conclusions in the perspective of modern plant sciences.

Keywords: Anesthesia, behavior chloroform, consciousness, ether, ethylene, photosynthesis, plants, plant movements, respiration, sensory systems

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Submitted: 10/15/2013

Revised: 12/30/2013

Accepted: 01/15/2014

Published Online: 01/29/2014

Citation: Grémiaux A, Yokawa K, Mancuso S, Baluška F. Plant anesthesia supports similarities between animals and plants: Claude Bernard's forgotten studies. Plant Signaling & Behavior 2014; 9:e27886; PMID: 24476640; http://dx.doi.org/10.4161/psb.27886

Anesthesia of animals and humans using volatile anesthetics

Oliver Wendell Holmes coined the term anesthesia in 1846. Anesthesia can be defined as loss of responsiveness to environmental stimuli. In humans and animals, it includes total lack of awareness. The term anesthesia is derived from the Greek word *anaisthēsia*, which means insensibility or inability to perceive (*aisthēsis* perception, *aisthanesthai* to perceive).

It is well established that animals can be anesthetized by inhalation of ether or chloroform vapors or by injection of ether – or chloroform-saturated solutions. This property of ether has been used since the middle of the 19th century and some of its derivatives are still used today to anaesthetize humans, in particular to prevent pain during surgery treatments. Depending on the quantity of volatile anesthetics metabolized by the animal, different stages of anesthesia were observed. Claude Bernard identified 3 stages.[2] In order to better understand the mechanism of the action of ether, Claude Bernard studied its effects under increased exposure time. At the first stage, the central nervous system was affected, the animal did not perceive pain or cold anymore, and it fell unconscious, but all the vital functions were preserved. At the second stage, the somato-sensory system was affected, so that the nervous system was disabled of sensation and the respiratory movements stopped. Finally, in the third and most advanced stage of anesthesia, the ability of cells to react to stimuli, what Claude Bernard called "irritability," was disrupted and the more reflexive actions such as heart beating and cilia movements were also stopped.

These experiments demonstrated that volatile anesthetics are not only acting on neurons but that they affect physiological processes in all cells. Thus, according Claude Bernard, sensation is not limited to organisms with a nervous system, but is a general property of cells and organisms.[1,2] Importantly, this general sensitivity of cells to anesthetics has recently been confirmed.[3,4] Different cells have different susceptibilities to volatile anesthetics, the neurons being the most sensitive. Interestingly in this respect, there are several similarities between plant cells and neurons.[5] To test the hypothesis that plants and animals have the same ability to sense stimuli, Claude Bernard was one of the first scientists to perform anesthesia on plants.[1,2]

Anesthesia of plants using volatile anesthetics

Claude Bernard was one of the first experimenters to characterize the effects of volatile anesthetics, such as ether and chloroform, on several processes in plants; including plant movements, seed germination, and photosynthesis.[1,2] In addition to germination and photosynthesis, the effects of anesthesia on respiration were also observed. The experimental methods used and the results obtained are presented in this section.

Plant movements

The leaves of *Mimosa pudica* present a long petiole supporting 1 or 2 close pairs of pinnae in their extremity, each pinna supporting about 20 leaflets. It is well known that in response to touch, electrical excitation or heat, the leaves of *M. pudica* exhibit rapid movements. The petiole and the pinnae fold down, and the leaflets fold inward, their up-sides getting close to each other. This movement involves specific organs, the pulvini, swelling at the bases of the petiole, pinnae, and leaflets. The stimulation of a leaf triggers a local modification of electrical potential, which starts a chain of reactions that lead to a modification of the turgor in the cells of the pulvini and, consequently, to changes in cell shape and volume (reviewed in refs. [6] and [7]). Depending on the stimulus, the stimuli can propagate to additional leaves. In addition, the leaves fold and unfold according to circadian cycle irrespectively of light status.

The experiment performed by Claude Bernard was simple. *M. pudica* plants were placed under a glass cloche together with a sponge dipped in ether under diffused light. Claude Bernard noticed that direct light increased the efficiency of anesthesia and could even kill the plants. Anesthetized plants temporarily lost their abilities to move in response to touch but they had demonstrated full recovery when the anesthetic agent was removed. Interestingly, Paul Bert, a student of Claude Bernard, remarked that even when the plant's sensitivity was disrupted, the circadian movements of leaves was not affected.[8] These observations indicated that anesthesia might affect plant ability to sense an external stimulus, but not its ability to move.

Seed germination

The germination of the water cress, *Lepidium sativum*, is known to be very fast and to take place when seeds are placed in 25–30 °C and high humidity for 2–3 d, which makes them an ideal model for research. Claude Bernard followed the effects of ether vapor on the germination of water cress seeds.[1] While seeds readily germinated in a control tube 2–3 d from the onset of the experiment, germination was interrupted in a tube containing ether. This interruption was reversible; if ether was removed after 4–5 d, germination restarted after 1 d. This experiment was repeated with identical results in cabbage, turnip, flax, and barley.

Photosynthesis

Finally, Claude Bernard studied the effect of volatile anesthetics on photosynthesis.[1] Aquatic plants *Potamogeton* and *Spirogyra* were placed in closed containers, containing water and carbon dioxide which was placed under direct light at 25–30 °C. In addition, a sponge dipped in distillated water and another one in ether-saturated water were placed into the control container and the test container, respectively. Then, the gas emitted by the preparation was collected and analyzed. The gas collected from the control container was rich in oxygen. In contrast, the gas from the ether container was rich in CO_2 and no oxygen was detected.[1] This finding showed that ether effectively inhibited photosynthesis, but without interrupting respiration processes.

Older and recent studies on sensitivity of plants to anesthetics

Claude Bernard was not the first one reporting on the sensitivity of plants to anesthetics. In 1847, Clemens had already reported that leaves of *Mimosa* and stamens of *Berberis* lost their sensitivities if exposed to vapors of ether. Similar studies with ether and chloroform, with the same outcome, were performed and published by Marcet in 1848, LeClerc in 1853, Pfeffer in 1873, Charles Darwin in 1875, Elfving in 1886, Haberlandt in 1890, and Francis Darwin in 1905.[9-18] Recent studies on *Mimosa* and *Dionea*, as well as on maize roots, confirm these early findings,[19-25] suggesting that animals and plants have similar sensory-motoric basis, which is sensitive to the same anesthetics,[26-28] as proposed by Claude Bernard, Charles Darwin, and Jagadish Chandra Bose more than 100 years ago.

Stressed plants synthesize anesthetics ether and ethylene

It is very interesting but almost never discussed in the literature that stressed plants produce not only plant-specific anesthetics ethylene, which is classified as plant hormone,[29-32] but also ether. Ethylene was used as powerful anesthetic in surgery[33] and plant cells synthesize ether under pathogen attack or wounding.[34-37] Intriguingly, ethylene is produced in stressed plants[29,30] and is also abundantly synthesized during, and necessary for, fruit maturation.[31,32] Ethylene effects on plant roots are similar to those induced by halothane[38] and anesthetized plants also showed reduced chilling injuries.[39] Moreover, anesthetics modulate seed germination[40] whereas only fully ripe fruits, obviously fully "anesthetized" with ethylene, are tasty and edible, whereas non-ripen fruits are usually less tasty and are often containing various toxic substances. Interestingly, fruit is the only plant organ evolved by flowering plants, without any breeding and human interventions, to be consumed by animals and humans in its living state.

Outlook

For Claude Bernard, the volatile anesthetics distinguished living organisms from "dead" organized matter.

He expressed this as: "What is alive must sense and can be anesthetized, the rest is dead." In effect, the volatile anesthetics affect the ability of cells to react in response to a stimulus. Neurons are very sensitive in this respect. Due to their specialization for integration of sensory information and high fidelity perception of the environment, neurons are the most sensitive to anesthesia. Similarly, plant cells are all excitable, and some of them are even specialized in perception, transmission, and integration of sensory information.[20-28] The knowledge accumulated during the last century shows that sensitivity to anesthetics is general phenomenon holding the key to the unity of life.[1,41,42]

However, as all cells have these sensory abilities, it is expected that they are all also susceptible to anesthesia, regardless of kingdom. In 1878, the ideas of Claude Bernard were new. According to him, the anesthetics distinguish the processes of organization and destruction since it affects, e.g., germination but not respiration, which is considered a chemical degradation. The influence of Linné's classification of species was still very strong, and the separation between plants and animal was very strict: the animal life was conceived to be based on senses and movements, but not that of plants. In this early period, Claude Bernard's pioneering experiments were the first to indicate that sessile plants have similar sensory systems as mobile animals. Since then, accumulated observations of plant ability to integrate complex stimuli have been well established.[27]

The effects of anesthetics are preeminent in excitable cells such as neurons, where the block of sodium and potassium channels dramatically affects their coherent oscillatory activity.[43,44] In plants, excitable cells are very abundant and sensitive, especially in the root apex, where a specific region, the transition or oscillatory zone, has been identified as a sort of "command center" showing intense[21] and coordinated oscillatory activities.[21,28] In animals, anesthetic-induced modification of ion channel activities was reported for the ligand-activated NMDA[45] and GABA channels.[46] Interestingly, plants also possess NMDA-like channels, usually referred to as glutamate-like receptors (GLRs),[47] whereas the GABA receptor remains elusive, having no conclusive role as of yet. Our recent results reveal that GLRs are highly expressed in the transition zone of the root, where they control endocytic vesicle recycling together with plant synaptotagmins (Matthias Weiland, Siao Wei, Stefano Mancuso, Frantisek Baluska, unpublished data). Based on the premise of a common mechanism of anesthetics in both plant and animal cells, a realistic scenario for its operation might involve similar modifications of endocytic vesicle recycling and ion channel activities.

Photosynthesis is regulated by opening or closing stomata.[48] Potassium channels are important not only for stomata movements but also play a role in plant organ movements.[43] Hence, it can be proposed that volatile anesthetics might act on potassium channels. Proven correct, such activity could explain why anesthesia disrupts photosynthesis and seed development but not respiration. More investigations of these rather neglected phenomena in plants are expected to reveal new fundamental biological processes relevant for both plants and animals.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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