

1 **Anesthetics, Anesthesia and Plants**

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13 **Abstract**

14 General anesthesia, its nature and how it exactly works, are still poorly understood. Plants

15 can also be anesthetized and lose their responses to external stimuli. Interestingly, plants

16 are known to produce endogenous anesthetic compounds to deal with stress. Plants offer

17 an excellent model object for studies on anesthetics and anesthesia.

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21 **Anesthetized plants**

22 General anesthesia has been used for the purpose of medical treatments since their effects
23 were coincidentally discovered about 200 years ago. Many attempts to give a scientific
24 explanation how these compounds function on nervous system have been made. However,
25 it is still a big mystery why so many different types of chemical compounds show the
26 same effect in humans and animals, loss of consciousness [1]. We recently reported that
27 general anesthesia does immobilize leaf-moving plants, such as *Mimosa pudica* or Venus
28 flytraps due to loss of action potentials [2]. Venus flytraps no longer generate electrical
29 signals and their leaf traps remained open when the trigger hairs were touched [2].
30 Moreover, pea tendrils stopped their circumnutation movements and were immobilized
31 in a curled shape [2]. The results of this study suggest that the lack of plant organ
32 movements is due to the inhibition of plant-specific action potentials. In addition, the
33 generation of reactive oxygen species and the disturbance of endocytic vesicle recycling
34 were also observed using Arabidopsis cells (Figure 1) [2]. We also found an impact of
35 general anesthesia on plant-specific events of development. Termination of seed
36 dormancy and biosynthesis of chlorophyll were strongly inhibited under anesthesia.

37 Importantly, the effects of anesthesia were reversible, as all these processes quickly
38 resumed immediately after anesthetic drugs were removed. Action potentials, reactive
39 oxygen species and endocytic vesicle recycling are common important cellular events
40 both in plants and animals and these were susceptible to anesthesia. The results suggest
41 that anesthetics could target similar cellular components shared by many living organisms
42 (figure 1 and see TEXT BOX). Therefore, we propose that plants can serve as model
43 organisms to study the underlying mechanisms to how anesthesia works, and may be
44 useful in unlocking the process in humans well after its first use almost 200 years ago.

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46 **Anesthetic actions in animals and plants**

47 Although the mechanism of anesthetics in living cells have been unclear, there were two
48 possible targets have been proposed in the research history, lipid membrane vs. specific
49 membrane protein. The former is well-known as the “Meyer-Overton theory of anesthesia”
50 that explains the correlation between the solubility of anesthetic molecules within lipid
51 membranes and their anesthetic properties [1]. The theory postulated that lipid-soluble
52 compounds could act as the anesthetics when these reach certain critical concentrations
53 in the membrane. After genetics emerged, many anesthesia studies have shifted their
54 focus to identify the specific protein targets of anesthetics. The favored model of

55 anesthetics actions is that they bind to neurotransmitter receptors, especially to glutamate
56 and GABA receptors. Intriguingly, the existence of plant-specific glutamate and GABA
57 receptors has emerged for signaling in control of growth, stress responses, and
58 development [7-10]. Similar to processes found in animals and humans, GABA lowers
59 and glutamate increases membrane excitability, but there is also a model in plants of
60 physical actions of anesthetics targeting membranes and electrons [1,11]. In addition,
61 voltage-gated ion channels are also proposed to act as the targets of anesthesia in animal
62 and human neurons [1]. Importantly, plants also express very similar channels. This
63 implicates that anesthetics could have the same targets in living cells.

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65 **Application of anesthesia for plant and general sciences**

66 In the early 1900s, anesthetic chloroform was once used by gardeners to force early
67 blooming of lilac flowers, to ensure that flowers were ready to be sold at Christmas.
68 Normally lilac flowers need a long winter phase to prepare for bloom and would only
69 flower in spring time. It appears that gardeners empirically knew that the treatment of
70 anesthesia shortens the period of “winter rest” and took advantage of it. In 1920, Indian
71 Sir J. C. Bose performed anesthesia on large trees with chloroform, allowing their
72 transplantation without serious damages to such trees. He used a huge tent to cover a

73 whole tree and filled the inside with chloroform. According to our recent report [2],
74 general anesthetics inhibit chlorophyll biosynthesis and the termination of seed dormancy.
75 Although the underlying mechanisms of these actions of anesthetic compounds are still
76 unclear, the sensitivity of plants to anesthetics could be useful in terms of the application
77 of drugs for research of plant sciences, or in the field of agriculture and horticulture.
78 Intriguingly, plants produce numerous endogenous anesthetics [3], especially when they
79 are stressed or wounded [3,6]. Of these anesthetics, ethylene and divinyl-ether are mainly
80 characterized as stress and dormancy hormones [4-6], but are rarely discussed from the
81 anesthetics point of view. It might be that signaling pathways connected to stress
82 responses, including pathogen attacks, will be sensitive to anesthetics. These are urgent
83 questions for further studies. Finally, plants can serve as model objects to unravel
84 mysteries of anesthesia in general and to advance the discovery and applications of
85 anesthetics in medicine.

86

87 **TEXT BOX**

88 French physiologist Claude Bernard performed many anesthetic experiments in the last
89 10 years of his life before his death in 1878 in Paris. He convincingly demonstrated that
90 leaves of *Mimosa pudica* were unresponsive upon touch stimuli under diethyl ether

91 atmosphere (Figure I from p. 259 in [12]). He performed many experiments in animals
92 and plants and arrived at the conclusion that ‘...plants and animals must share common
93 biological essence that must be disrupted by anesthetics.’ [12]. Our recent report on
94 anesthesia and plants was conducted based on Bernard’s work [2].

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96 **References**

- 97 [1] Rinaldi, A. (2014) Reawakening anaesthesia research. *EMBO Rep.* 15, 1113–1118
- 98 [2] Yokawa, K. et al. (2018) Anesthetics stop diverse plant organ movements, affect
99 endocytic vesicle recycling, ROS homeostasis, and block action potentials in Venus
100 flytraps. *Ann. Bot.* mcx155, DOI: 10.1093/aob/mcx155
- 101 [3] Tsuchiya, H. (2017) Anesthetic agents of plant origin: a review of phytochemicals
102 with anesthetic activity. *Molecules* DOI: 10.3390/molecules22081369
- 103 [4] Stumpe, M. et al. (2008) Divinyl ether synthesis in garlic bulbs. *J. Exp. Bot.* 59, 907–
104 915
- 105 [5] Taylorson, R. B. (1982) Anesthetic effects on secondary dormancy and phytochrome
106 responses in *Setaria faberi* seeds. *Plant Physiol.* 70, 882–806
- 107 [6] Baluška, F. et al. (2016) Understanding of anesthesia – why consciousness is essential
108 for life and not based on genes. *Commun. Integr. Biol.* 9, DOI:

109 10.1080/19420889.2016.1238118

110 [7] Mousavi, S. A. et al. (2013) GLUTAMATE RECEPTOR-LIKE genes mediate leaf-
111 to-leaf wound signalling. *Nature* 500, 422–426

112 [8] Žárský, V. (2015) Signal transduction: GABA receptor found in plants. *Nat. Plants* 1,
113 15115

114 [9] Weiland, M. et al. (2016) Signalling via glutamate and GLRs in *Arabidopsis thaliana*.
115 *Funct. Plant Biol.* 43, 1–25

116 [10] Ramesh, S. A. et al. (2017) γ -Aminobutyric acid (GABA) signalling in plants. *Cell*.
117 *Mol. Life Sci.* 74, 1577–1603

118 [11] Turin, L. et al. (2014) Electron spin changes during general anesthesia in *Drosophila*.
119 *Proc. Natl. Acad. Sci. USA* 111, E3524–E3533

120 [12] Bernard, C. (1878) *Leçons sur les phénomènes de la vie communs aux animaux et*
121 *aux végétaux*, Librairie J-B. Baillière et Fils

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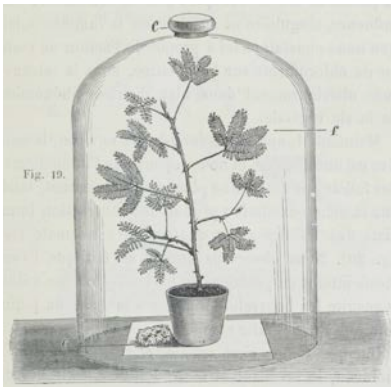
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127 **Figure legends**



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129 Figure I. Bernard's ether experiment. Anesthetized mimosa plant with ether-containing

130 sponge.

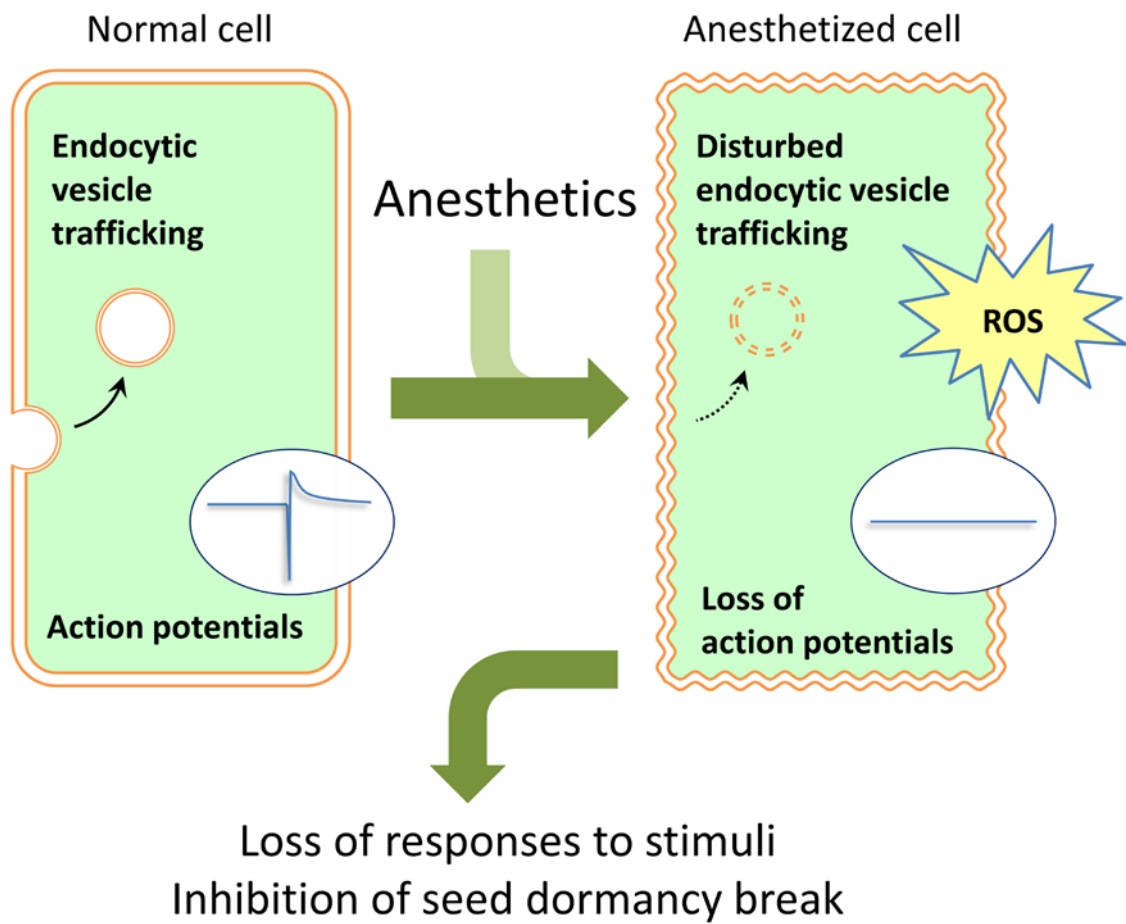
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138 Figure 1. A proposed model of a plant cell under anesthesia. Anesthetics block plant

139 action potentials. Membrane trafficking is disturbed and excessive reactive oxygen

140 species (ROS) are generated rapidly [2]. These quick cellular responses lead to the loss

141 of response to external stimuli and inhibition of break of seed dormancy. Double-line

142 depicts plasma membrane of cell, compromised in cells under anesthesia.