



To flourish or perish: evolutionary TRiPs into the sensory biology of plant-herbivore interactions

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Abstract

The interactions between plants and their herbivores are highly complex systems generating on one side an extraordinary diversity of plant protection mechanisms and on the other side sophisticated consumer feeding strategies. Herbivores have evolved complex, integrative sensory systems that allow them to distinguish between food sources having mere bad flavors from the actually toxic ones. These systems are based on the senses of taste, olfaction and somatosensation in the oral and nasal cavities, and on post-ingestive chemosensory mechanisms. The potential ability of plant defensive chemical traits to induce tissue damage in foragers is mainly encoded in the latter through chemesthetic sensations such as burning, pain, itch, irritation, tingling, and numbness, all of which induce innate aversive behavioral responses. Here, we discuss the involvement of transient receptor potential (TRP) channels in the chemosensory mechanisms that are at the core of complex and fascinating plant-herbivore ecological networks. We review how “sensory” TRPs are activated by a myriad of plant-derived compounds, leading to cation influx, membrane depolarization, and excitation of sensory nerve fibers of the oronasal cavities in mammals and bitter-sensing cells in insects. We also illustrate how TRP channel expression patterns and functionalities vary between species, leading to intriguing evolutionary adaptations to the specific habitats and life cycles of individual organisms.

Keywords TRP channels · Herbivores · Plant defenses · Noxious chemicals

Introduction

Plants have evolved multiple detection and defense mechanisms to withstand extensive tissue damage triggered by herbivores. Many species have developed tolerance to their consumers by increasing photosynthetic rate, growing compensatory tissues, and mobilizing resources from roots to shoots [201]. Another strategy involves production of specialized chemicals that induce repellent, toxic, and/or antinutritional effects in the herbivores. These compounds can be divided into several classes depending on their chemical properties and include alkaloids, terpenoids, phenolics, sulfur-containing compounds, proteinase, and growth inhibitors. On the other

hand, as herbivores clearly cannot reject all food sources, they co-evolved complex protective mechanisms to avoid or resist the plants defensive traits. While plant-derived protective chemicals can cause serious negative effects such as liver damage, heart failure, or even death, herbivores may still consume them because of dietary superiority, palatability, or even addictive properties. For instance, locoweed (crazyweed) or larkspur plants are vastly consumed by grazing livestock due to their large protein content and pleasant taste [41]. However, overconsumption generates a variety of symptoms, including visual impairment, trembling, and neurological damage. Although intoxication of wild animals, such as deer, could be explained by a change of perception when starved, triggering consumption of more palatable species, the livestock behavior is an example of pure addiction to plant-induced psychoactive effect. Evidently, herbivores evolved complex sensory mechanisms to avoid intoxication. Essentially, taste, olfaction, and somatosensation in the oral and nasal cavities, as well as post-oral chemosensory mechanisms in the digestive tract serve to detect and avoid potentially toxic plants. In general, animals are attracted to sweet, floral fragrances, and flavors and repelled by bitter and pungent traits. Since many

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unpleasant, bitter-tasting plants are not harmful, it is essential to distinguish between mere bad taste and chemesthetic sensations such as heat, cold, pain, irritation, tingling, or numbness. The latter sensations are largely mediated by modulation of ion flow via voltage-gated Na^+ and K^+ channels, whose activation sustains the development and extinction of the action potential in somatosensory neurons, and via acid-sensing ion channels (ASIC) and transient receptor potential (TRP) channels, whose activation produces cationic influx, and an associated depolarization that initiates and/or modulates action potential firing. Because of their rising relevance for the understanding of the mechanisms of chemosensation, in this review, we will focus on the ecological role of TRP channels as mediators of multiple interactions between different classes of plant defensive chemicals and herbivores (Table 1).

Olfaction, gustation, and chemesthesis—detection frontiers

The sense of olfaction serves to the detection of airborne chemicals and in most vertebrates is mediated by two distinct systems. The main system perceives airborne chemicals and the accessory system detects fluid-phase chemicals. Olfaction arises once odorant molecules bind to the olfactory (odorant) receptors (OR) located on the cilia of olfactory neurons in the epithelium at the roof of the nasal cavity (Fig. 1; [177]).

The OR gene superfamily is one of the largest in the human genome comprising 390 putatively functional genes and 465 pseudogenes organized into 18 gene families and 300 subfamilies [167]. It covers up to 3% of the human genome, illustrating their critical function in mammalian physiology [179]. The ratios of gene to pseudogene are highest in rodents and lowest in human, indicative of a greater evolutionary pressure for olfaction in the rodent survival. Allelic exclusion of OR genes causes expression of typically one odorant receptor allele in each sensory neuron, although the mechanism underlying this process is still not fully understood [167]. ORs are activated in different combinations, allowing multitude odorants discrimination [96].

Taste is the sensory impression of the food or other substances on the tongue and in the soft tissues of the mouth. The five basic tastes modalities, recognized by humans as well as most animals are bitter, salty, sour, sweet, and umami, all giving information on the nutrient composition of food and helping to prevent ingestion of toxic and harmful products. The taste buds are the basic anatomical structures detecting tastes, located in the stratified epithelium of the tongue, palate, pharynx, larynx, and epiglottis [177]. In humans, most of the taste buds are situated at the back of the tongue with the number that varies significantly between different people (Fig. 1c) [98, 177]. Taste receptor cells (TRCs) can be divided into three types, each responding to diverse gustatory

stimulation. In contrast, the so-called “chemesthesis” (also known as trigeminality or general chemical sense) is related to the chemical activation of sensory nerves resulting in touch and thermal sensations, as well as a wide variety of other sensations such as tingling, itch, numbness, and pain [116]. Chemesthesis (Fig. 2) is associated with nerve fibers originating from the trigeminal (TG) and dorsal root ganglia (DRG) [193, 194] and their stimulation by different chemicals induces pungent sensation as burning, tingling, or cooling also in absence of an olfactory stimulation [116].

Most odorants at sufficient concentrations stimulate TG nerve endings in addition to the olfactory nerve [86, 199]. Trigeminal chemosensory nerve endings in the nasal and oral cavity are a first recognition mechanism protecting from noxious stimuli, and these present in the mouth also enhance the flavor. Nociceptive TG neurons can be stimulated by a large group of chemicals classified as irritants, including natural plant compounds, air pollutants, and endogenous substances. It has been clearly established that several members of the TRP protein superfamily are essential for olfaction, taste, thermo-, osmo-, mechano-, and chemosensation (Fig. 3) [24, 39, 94, 154, 156, 176, 186].

Transient receptor potential cation channels

TRPs are cation channels, with variable permeability to Ca^{2+} and Mg^{2+} , ranging from Ca^{2+} impermeable (TRPM4 and TRPM5 are only permeable to monovalent cations), over non-selective Ca^{2+} -permeable (most TRP channels) to highly Ca^{2+} selective (TRPV5 and TRPV6) [125, 157, 158]. Many TRPs are able to conduct other divalent cations such as Ba^{2+} , Cd^{2+} , Co^{2+} , Mn^{2+} , or Zn^{2+} [27]. The cation permeability of TRP channels highly depends on the pore structure. The structure-function analysis of TRP pores revealed essential residues of the selectivity filter necessary to distinguish between different ions. For instance, neutralization of D546 of TRPV1 and the corresponding 682 of TRPV4 reduces their Ca^{2+} and Mg^{2+} conductance. A further mutation of D672 amino acid of TRPV4 promotes additional selectivity decrease for divalent cations and changes the monovalent permeability [170].

TRP channels are expressed in numerous tissues and cell types, and exhibit great diversity in activation mechanisms [40, 145, 176, 215]. They are formed by homo- or heteromultimerization of four subunits consisting of six transmembrane (TM) segments with the cationic pore loop located between fifth and sixth TM segments (Fig. 3). The carboxyl-(C)- and amino-(N)-termini are located intracellularly and are involved in the mechanisms of channel opening and closing (gating), enzymatic activity, and interactions with many proteins through various residues or domains, as for instance ankyrin (ANK) repeats [64, 156].

Plant secondary metabolites—elaborate chemical defense system

Alkaloids—psychoactive modulators

Alkaloids such as caffeine, nicotine, strychnine, cocaine, and quinine are widely produced by many plants. They function as insect repellents and vertebrate deterrents by inducing neurotoxic effects due to their functional similarity with endogenous neurotransmitters. For that reason, they often induce drug addiction in animals, as for instance, wallabies search for opioid containing poppy plants [43], dogs learn to harass certain toads releasing hallucinogen bufotenin [42], and dolphins eat toxic pufferfish [161]. Drug dependence is mediated by cortico-mesolimbic dopaminergic system and reward/pleasure pathways and modifies neuronal signaling pathways in the brain, which in turn alters behaviors. It has been shown that members of the canonical (TRPC) and vanilloid (TRPV) subfamilies of TRP channels are involved in drug abuse due to their function in psychostimulant-induced behavioral and neuronal plasticity [222].

Plants from the *Nicotiana* genus produce nicotine (3-[(2S)-1-methylpyrrolidin-2-yl]pyridine) in the roots as a response to leaf wounding (Fig. 4). This alkaloid activates nicotinic acetylcholine receptors (nAChRs) in plant consumers, influencing sensory and motor functions controlled by the cholinergic pathway [45, 57, 120].

One of the best examples is a role of TRPCs in nicotine-induced behaviors of *Caenorhabditis elegans* [63]. This nematode expresses TRP-1 and TRP-2 (TRPC homologs) in multiple types of neurons [226, 229], acting downstream of nAChRs signaling pathway involved in a variety of responses to nicotine, such as stimulated crawl-speed followed by adaptation to this chemical. When nicotine addicted *C. elegans* were placed in nicotine-free environment they presented withdrawal symptoms, where the worms became dependent on nicotine for their normal locomotion patterns [63]. Moreover, *C. elegans* worms having null mutations in *trp-1* and *trp-2* display reduced behavioral responses to nicotine. Although the precise mechanism is not clear it was proposed that TRP-2 acts as a receptor-operated cation channel coupled to the Gq-PLC β pathway. This signaling is a common pathway in mammalian responses to nicotine, suggesting evolutionarily conserved role of TRPC channels and nAChRs in nicotine sensing.

Cholinergic neurons and nAChRs also play a role in learning and memory pathways, mood and processing of painful stimuli [143]. Because of its toxicity, nicotine has been used historically in agriculture to control pests. However, its high toxicity in mammals, short life span, and shallow insecticide efficiency (acting only on soft bodied insects with piercing mouth apparatus and mites) nicotine use has been limited. Nicotine poisoning produces various symptoms related to malfunction of the central nervous system. For example, nicotine intoxication of honeybees induces ascending motor paralysis

of the nerve cord resulting in complete body paralysis with occasional twitching of tarsus, antenna, or abdomen [139]. The pungency of nicotine was originally related to a specific activation of nAChRs present in nociceptive fibers [57, 120, 195, 196]. However, nicotine was later found to activate mouse and human TRPA1 channels, to activate mouse sensory neurons, and to trigger ventilatory responses in mice in a TRPA1-dependent manner [57, 206]. Interestingly, nicotine was shown to have a relatively slow but sustained stimulatory effect on TRPA1 (Fig. 5a), which contrast with the rapidly-installing and quickly desensitizing action on nAChRs. Furthermore, nicotine induced two types of intracellular Ca²⁺ responses in mouse trigeminal ganglion neurons: rapid and quickly desensitizing ones mediated by nAChRs and slower, more sustained ones mediated by TRPA1. It could be therefore concluded that nAChRs and TRPA1 confer sensory neurons the ability to respond to nicotine in a wide range of concentrations over prolonged periods. Of note, nicotine has a bimodal effect on mouse TRPA1, activating when applied in low micromolar concentrations and inhibiting in the millimolar range (Fig. 5b). However, the physiological relevance of the inhibitory effect is not yet clear. Taken together, these findings indicate that the irritating effects of nicotine in mammals may be attributed to nAChRs and TRPA1 activation. This further supported the already existing notion that TRPA1 mediates the detection of chemicals such as isothiocyanates, caffeine, and phenols, whose production in plants is enhanced upon herbivore attack.

The tobacco specialist *Manduca sexta* (tobacco hornworm) tolerates nicotine at doses (LD₅₀ 1.5 g/kg BW) that are fatal to other herbivores (LD₅₀ for mice 0.0003 g/kg BW, birds 0.0178 g/kg BW, and humans 0.002 g/kg BW) [223] and is less prone to parasitoids when fed with a nicotine-rich diet [19]. The larvae cope with high doses of nicotine via at least three different strategies. First, after absorption from food, the alkaloid is transported via hemolymph and excreted with feces by Malpighian tubes containing specific ABC transporters [11, 114, 133]. Second, nicotine is metabolized in the neural sheath. Third, it has been proposed that *M. sexta* larval neurons are less sensitive to nicotine when compared to those of other animals. This change in sensitivity could be attributed to the insect nervous tissue, which is protected by a very efficient ion-impermeable sheath, or to a distinct composition of nAChR subunits and/or different TRPA1 activation properties. It has been shown that the genome of *M. sexta* encodes a single *TrpA1* gene and that the protein is expressed in the lateral and medial styloconic sensilla [2]. It was proposed that MsexTrpA1 functions as a molecular integrator of chemical and thermal input in gustatory receptor neurons (GRNs) stimulated by aristolochic acid, a bitter compound produced by the plant family *Aristolochiaceae* (birthworts).

Caffeine (1,3,7-trimethylxanthine), a drug of choice for many, is another alkaloid produced by many plants with an associated protective function against herbivores (Fig. 6a).

Table 1 Summary of the discussed compound activity on the TRP channels

Plant genus	Compound name	Cellular targets	Reference	Response
<i>Nicotiana</i> <i>Solanum</i> <i>Lycopersicon</i> <i>Brassica</i> <i>Apium</i>	Nicotine	TRP-1 TRP-2 TRPA1	[57, 63, 206]	Influences behavioral and motor function
<i>Coffea</i> <i>Camellia</i> <i>Cola</i> <i>Citrus</i> <i>Ilex</i> <i>Paullinia</i>	Caffeine	TRPA1	[122, 146, 149, 150]	Behavioral alteration, memory enhancement, cardiovascular and respiratory effects
<i>Cannabis</i>	Δ^9 -Tetrahydrocannabinol Tetrahydrocannabinolic acid Cannabinol Cannabichromene Cannabigerol Cannabidiol	TRPA1 TRPV1 TRPM8	[49, 51, 92, 124, 234]	Psychoactive or non-psychoactive, motor and behavioral function, antinociception, antihyperalgesia
<i>Capsicum</i>	Capsaicin	TRPV1	[33, 46, 72, 75, 93, 119, 162]	Respiratory and GI effect, irritant (skin, eyes, mouth), analgesic and anti-inflammatory
<i>Vanilla</i>	Vanillin	TRPV1 TRPA1 TRPV3	[130, 227]	Behavioral alterations, allergen, irritant
<i>Syzygium</i> <i>Cinnamomu</i> , <i>Myristica</i> <i>Ocimum</i> <i>Zingiber</i> <i>Vanilla</i> <i>Laurus</i> <i>Zingiber</i>	Eugenol	TRPV1 TRPA1 TRPM8	[17, 38, 105, 121, 173, 174, 227, 230]	Behavioral alterations, allergen, respiratory effects, analgesic and anti-inflammatory
<i>Piper</i>	Zingerone	TRPV1 TRPA1	[72, 112, 127]	GI effects, antimicrobial
<i>Piper</i>	Piperine Isopiperine isochavicine Piperanine Piperolein N-tetra	TRPV1 TRPA1	[140, 166]	Respiratory effects, antioxidant, anti-inflammatory, and antimicrobial agent
<i>Brassica</i> <i>Raphanus</i> <i>Armoracia</i>	Allyl isothiocyanate	TRPA1 TRPV1	[5, 20, 61, 73, 164]	Respiratory effects, irritant, cytotoxic, antimicrobial
<i>Allium</i>	Diallyl sulfide Diallyl disulfide Diallyl trisulfide Allicin	TRPA1 TRPV1	[20, 109, 132, 187]	Respiratory, GI and irritant effects, genotoxic, cytotoxic
<i>Satyrrium</i> <i>Mimulus</i>	Linalool Eucalyptol Elemicin α -pinene Myrcene Limonene	TRPA1 TRPM8	[21, 118, 165, 185]	Repellent or attractant action, analgesic, antimicrobial, anti-inflammatory, antinociception
<i>Corymbia</i> <i>Cymbopogon</i> <i>Java</i>	Citronellal Citronellol Citral Geraniol	TRPA1 TRPV1 TRPM8 TRPV3	[76, 178, 200]	Repellent or attractant action, analgesic, antiseptic, antimicrobial, anti-inflammatory, antinociception

Caffeine is biosynthesized from the plant nucleotide xanthosine in a four-step sequence involving three methylations (Fig. 6b) [14, 53]. Few ecological theories exist to explain caffeine

function in plants. The autotoxic theory claims that caffeine, released from leaves and seeds falling into the ground, inhibits germination and growth of other plants around the coffee

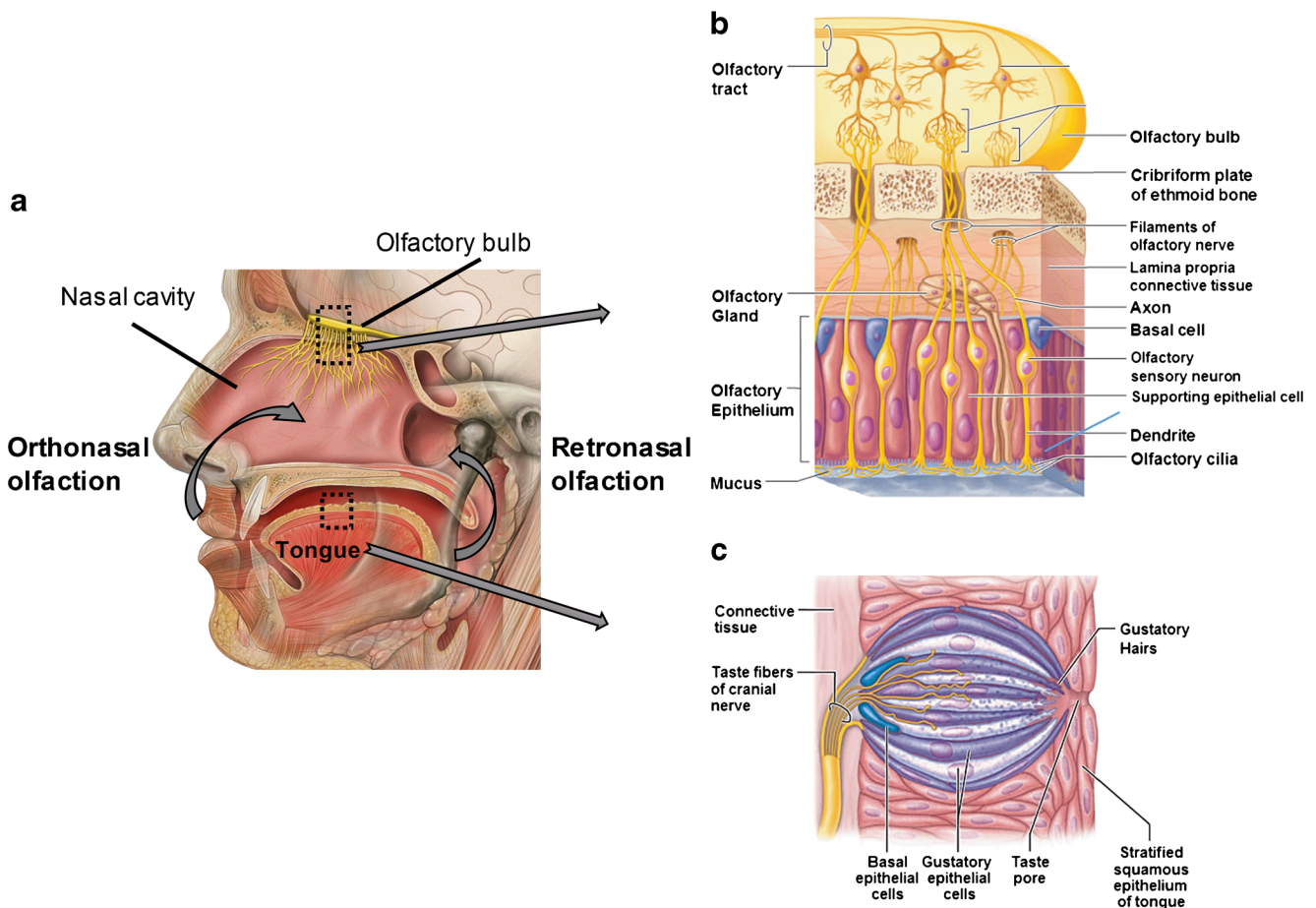


Fig. 1 Human olfaction and gustation. **a** Localization of olfactory epithelium covering the superior nasal concha and directions of air entering nasal cavity. Modified from P.J. Lynch and C. Jaffe. **b** Schematic representation of laminar and circuit organization of the olfactory bulb. Olfactory bipolar neurons, located in the olfactory epithelium, contain 5 to 20 hair-like cilia trapping odorant molecules. Neurons are surrounded by columnar supporting cells, containing yellow-brown pigment, giving them yellow tint. Together with olfactory glands, supporting cells produce mucus that helps to dissolve and detect airborne chemicals. Olfactory stimulation is the only sensory information

plants. Exogenously applied caffeine affects metabolic pathways as well as can inhibit cell division. Secondly, the chemical defense theory postulates a protective effect of caffeine against the fungus *Gibberella xylarioides* [79] and against herbivores. Caffeine sprays prevent feeding of tobacco horn-worms or snails [83] and inhibit enzymes in the insect nervous system causing paralysis and death. Caffeine can produce more intriguing behavioral effects in insects, such as construction of spider webs with no symmetry [221] and drowning of mosquitoes larvae. Interestingly, some *Citrus* flowers contain caffeine to protect them from its main pathogens, e.g., *Hemileia vastatrix*, *Colletotrichum kahawae*, and low, non-repellent caffeine concentrations (below 0.058 mg/ml) in their nectars, attracting pollinating honeybees [225]. Bees detect caffeine with neurons located in sensilla of the mouthparts, which express HsTRPA [108], a member of the TRPA channel subfamily, which may

that directly reaches the cerebral cortex, while other sensations are conveyed through the thalamus. **c** Structure of human taste buds. Taste buds are located along the edges of upper surface of the tongue and palate in different types of papillae. They are flask-shaped consisting of 50 to 150 cells (gustatory or basal cells). Gustatory hairs, which extend through the taste pore from the gustatory cells, are sensitive to chemicals present in the saliva. Sensory dendrites present around the gustatory cells receive signals from several receptor cells and conduct a signal to brain. Modified from Marieb and Hoehn [137]

serve as sensor for this bitter chemical [95]. Caffeinated honeybees show altered pollinating behavior, with increased recognition of caffeine containing nectars, suggesting a chemical-induced enhancement of the insect's memory [225].

This is beneficial for both species, as plants ensure pollinations and bees may locate and revisit their food sources more easily. In birds, exposure to caffeine doses below 30 mg/kg causes several cardiovascular and respiratory effects, inducing more awakens and alertness and enhancement of their productivity [70]. Higher doses are highly toxic, causing seizures and death. In larger animals, caffeine can induce symptoms as hyperactivity, restlessness, vomiting, an elevated heart rate and hypertension, seizures, and finally death. For humans, the lethal caffeine dose is approximately 10 g if taken at once, which corresponds to roughly 105 cups of coffee. Several mechanisms are responsible for caffeine's pharmacological and toxic

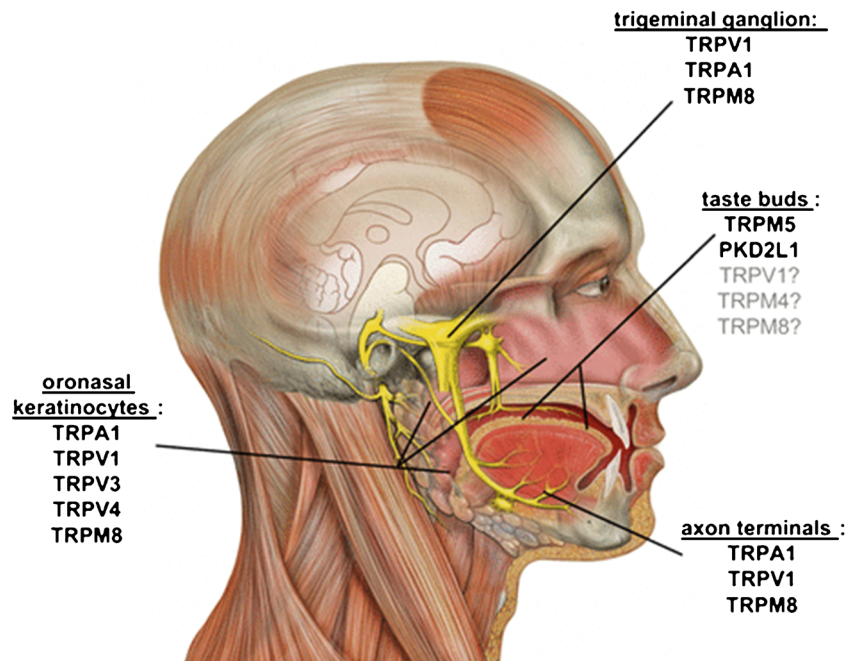


Fig. 2 Involvement of the mammalian TRP channels in chemesthesis and taste. Chemesthesis comprises a broad range of sensations, associated with diverse mechanisms present in a wide array of sensory structures, including nociceptors, other free nerve endings, and keratinocytes. Oronasal chemesthetic signals are conducted by somatosensory fibers in the trigeminal (V), glossopharyngeal (IX), and vagus (X) nerves [186]. Sensory neurons and oronasal keratinocytes express multiple TRP channels implied in chemesthesis and gustation. Another member of the TRP

superfamily, TRPM5, has been identified in the subset of taste bud cells (type II) that to function in signal transduction for sweet, bitter, and umami taste [181, 182]. The molecular mechanism of sensing these tastes involves activation of G protein-coupled receptors (GPCRs), followed by IP₃-mediated release of Ca²⁺ from intracellular stores resulting in Ca²⁺-induced activation of TRPM5 [126, 180, 182]. Reproduced with permission from Roper [186]

effects. For instance, activation of ryanodine receptors (RyRs) leading to the emptying of intracellular Ca²⁺ stores represents a well-known and widely studied effect [214, 230]. Caffeine also inhibits phosphodiesterases increasing cAMP, thereby causing cardiovascular modulation [59], and blocks adenosine receptors playing key roles in behavioral and cognitive functions [34]. Mouse TRPA1 is rapidly activated by caffeine in transfected cells as well as in capsaicin-insensitive DRG neurons. Neurons from animals lacking TRPA1 are deficient in rapid responses to caffeine and only exhibit a slower intracellular Ca²⁺ increase that may be attributed to RyR receptors activity [150]. Wild-type mice show highly aversive behavior towards caffeine in drinking water, but this aversion is much less pronounced in mice not expressing TRPA1. This raises the possibility that the detection of this compound occurs via a trigeminal-dependent pathway. Interestingly, caffeine has a suppressing action on human TRPA1, and mutation of the amino acid residue methionine in the position 268 to proline was shown to be responsible for the differences between species (Fig. 7) [149]. Since the M268 residue is conserved in rodents, it is possible that, in addition to its bitterness, caffeine has also a pungent character that may enhance its ability to evoke aversive behavior in these animals. Conversely, the presence of a proline at this position in primate TRPA1 may allow intake of caffeine, with the consequent beneficial

pharmacological effects. The bitter perception of caffeine in mammals is attributed to the family of taste receptors that are evolutionary similar to insect gustatory receptors [36, 37, 181].

In insects, TRPA1 was found to be expressed in bitter-sensing GRNs responsible for aversion behaviors. Although this channel is not directly activated by caffeine, it is clear that it contributes to aversive behavior in insects. In *Drosophila*, several gustatory receptors such as Gr66a, Gr93a, and Gr32a are implicated in caffeine-evoked action potentials [122, 146]. Accordingly, aversive behavior towards the bitter aristolochic acid was reported to be TRPA1-dependent. However, dTRPA1 is not directly activated by aristolochic acid, but via a phospholipase C (PLC) signaling cascade [104].

Cannabinoids—addictive guardians

The *Cannabis* genus contains multiple species of flowering plants as *Cannabis sativa*, *Cannabis ruderalis*, or *Cannabis indica*, producing more than 100 different cannabinoids along with the primary psychoactive component, Δ⁹-tetrahydrocannabinol (Δ⁹-THC) [9, 198]. These compounds ensure plant survival in many different climates, from hot, tropical regions to very cold and harsh conditions in mountains. Cannabinoids such as tetrahydrocannabinolic acid (THCA) are toxic to cells and are produced in the extracellular compartment of the

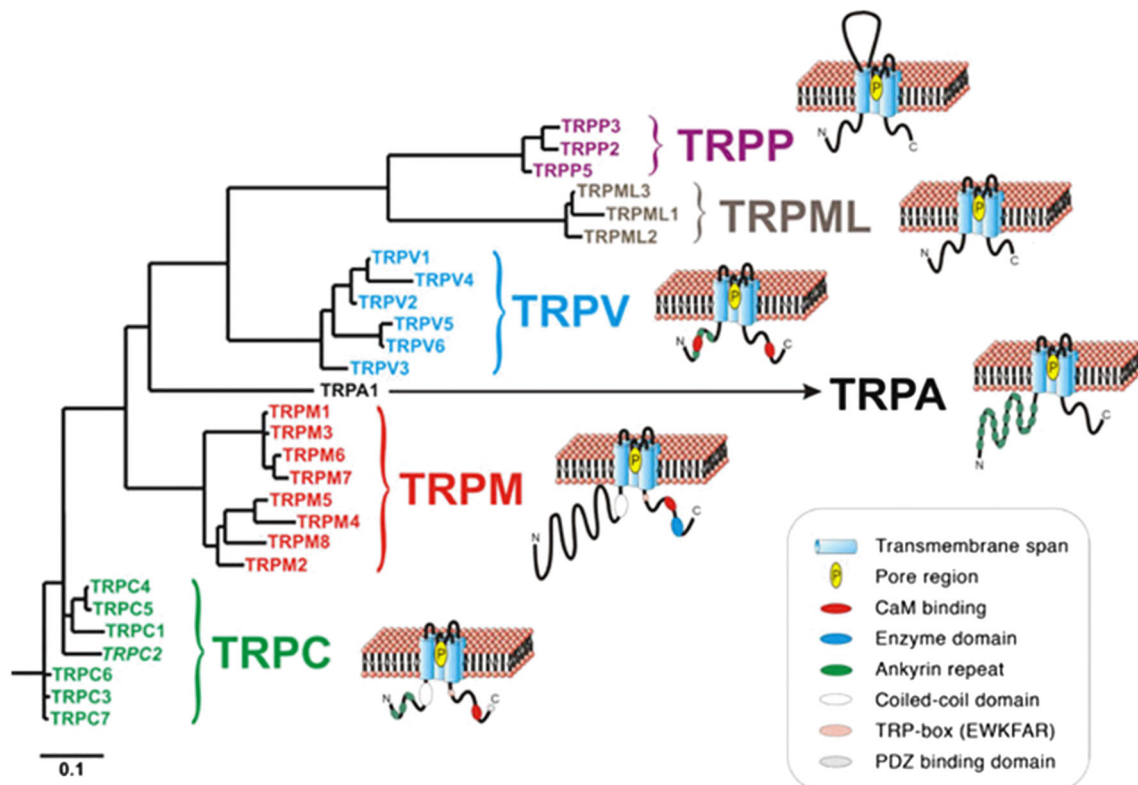


Fig. 3 The phylogenetic tree and main structural features of mammalian TRP channels. TRPs can be classified into six subgroups containing TRPA (ankyrin), TRPC (canonical), TRPM (melastatin), TRPV (vanilloid), TRPP (polycystin), and TRPML (mucolipin) [155, 156].

trichomes, hair-like growths on the plant leaves and buds [9], where they protect plant tissues from insects and herbivores [197]. THCA, together with other chemicals including terpenes and flavonoids, cause cannabis plant extracts to have bitter, unpleasant taste, strong aroma, as well as antimicrobial activity [12]. THCA is a precursor of Δ^9 -THC, which is formed during progressive decarboxylation in the course of drying process [81]. In contrast to Δ^9 -THC, THCA is a non-psychoactive chemical that has been found to have multiple health benefits. Consumption of THC may induce hyperemesis syndrome, impaired coordination and performance, anxiety, suicidal ideations/tendencies, and psychotic symptoms [117, 198]. Cannabinoids actions have been attributed to activation of metabotropic cannabinoid receptors CB1 and CB2. These receptors are present in the central and peripheral nervous system and are endogenously activated by endocannabinoid neurotransmitters such as N-arachidonylethanolamine (anandamide) and sn-2-arachidonoylglycerol (2-AG) [198]. The endocannabinoid system (ECS) is involved in many physiological and cognitive processes, including appetite, pain, mood, and memory. The ECS functions in exercise-induced euphoria, as well as in the modulation of locomotor activity and motivational salience for rewards. Thus, due to its structural similarity to ECS signaling molecules, Δ^9 -THC mimics rewards correlated with fitness, which may explain the addictive effects of the compound [203].

Seventh subgroup contains non-mammalian, TRPN (NOMPC-like), expressed in fish [192] and invertebrates [218]. All TRP channels have an ancient origin related to TRPY subfamily of fungi [23, 52, 171]. Reproduced with permission from Nilius et al. [159]

Further, cannabinoids induce supraspinal, spinal, and peripheral antinociception and antihyperalgesia in different pain syndromes [30, 31, 44]. Moreover, chemicals belonging to the aminoalkylindole family of cannabinoids produce peripherally mediated antinociception in acute pain models [135] and alleviate hyperalgesia/allodynia induced by capsaicin [82, 89], heat [90], inflammation [148, 234], and nerve injury [65, 184, 227]. Δ^9 -THC and cannabidiol were shown to relax hepatic and mesenteric arteries, via activation of CGRP-containing sensory nerve endings that innervate the vascular smooth muscle. This effect was independent of cannabinoid receptors and TRPV1 [235]. The population of capsaicin-sensitive TG neurons was responsive to both AITC and Δ^9 -THC, suggesting a contribution of TRPA1 to cannabinoid-induced inflammatory hypersensitivity and vasodilation [92].

Phytocannabinoids such as cannabichromene (CBC), cannabigerol (CBG), cannabidiol (CBD), Δ^9 -THC, THCA, or CBDA were shown to influence the TRPA1- and TRPM8-mediated elevation of Ca^{2+} in DRG sensory neurons and in an overexpression system [49, 51]. The potency of these compounds followed the strength of their electrophilic nature, suggesting the formation of covalent bonds with cysteine residues as underlying mechanism of channel activation [51, 80, 131]. Although, cells expressing TRPA1 responded to multiple phytocannabinoids, the responses in TRPA1 expressing DRG

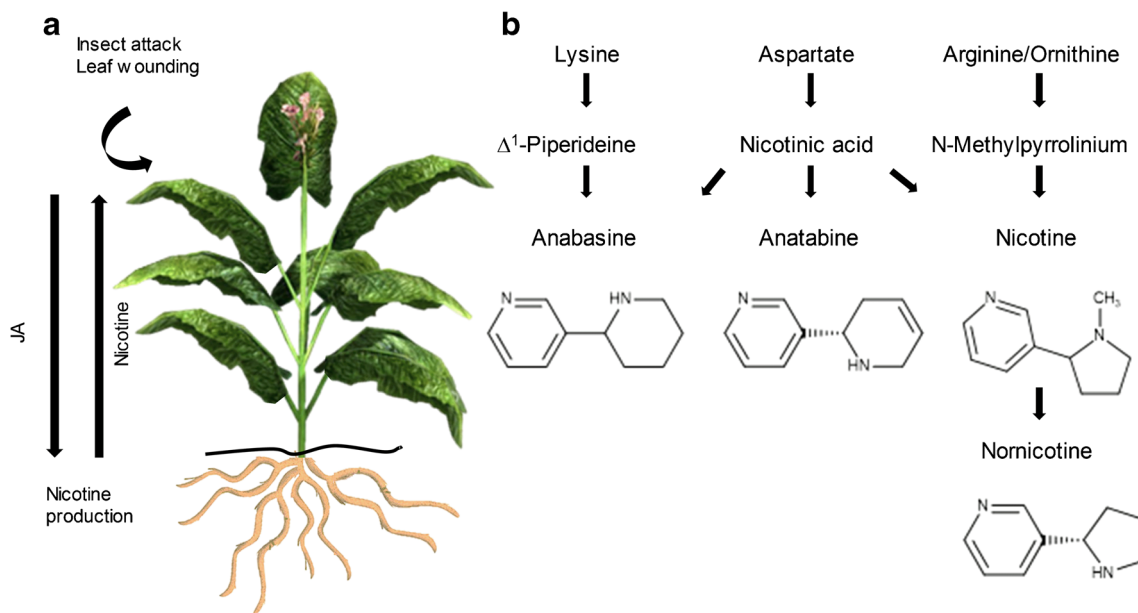


Fig. 4 Nicotine synthesis in plants. **a** Tissue damage caused by herbivory attack enhances the production of the plant phytohormone jasmonate (JA), which is transported into the roots, activating nicotine synthesis by condensation of the methylpyrrolinium cation derived from putrescine and nicotinic acid [100, 101]. Nicotine is then transported to leaves,

where is deposited into vacuoles. **b** Simplified biosynthesis diagram of the four main tobacco alkaloids. Diverse amino acids are used as building blocks in the synthesis of the pyridine, pyrrolidine, and piperidine rings, required to produce nicotine, nornicotine, anabasine, and anatabine [29, 100]

neurons were lower. This could be attributed to the lower expression levels in comparison with the overexpression system, as well presence of other channels. Both human and rat recombinant TRPV1 were shown to be insensitive to most of phytocannabinoids, with only CBD, CBG, and CBDA being TRPV1 activators [124]. On the other hand, pre-incubation with CBC and CBG inhibited Ca^{2+} elevation in cells overexpressing TRPA1 and in DRG neurons. Moreover, in TG neurons, the synthetic cannabinoids WIN and AM1241 inhibit the responses to capsaicin and AITC, via activation of TRPA1 expressed in the sensory neurons [3]. Finally, TRPM8

responses to menthol and icillin were inhibited by some phytocannabinoids [51], as well as endocannabinoids such as anandamide and N-arachidonoyldopamine [49, 50].

Potential roles of reactive oxygen and nitrogen species

Many *Cannabis*, *Solanum*, and *Arabidopsis* plants are able to generate hydrogen peroxide (H_2O_2), in response to plant wounding by herbivores and pathogens. The production of reactive oxygen species (ROS) such as O_2^- , OH^- , or H_2O_2 ,

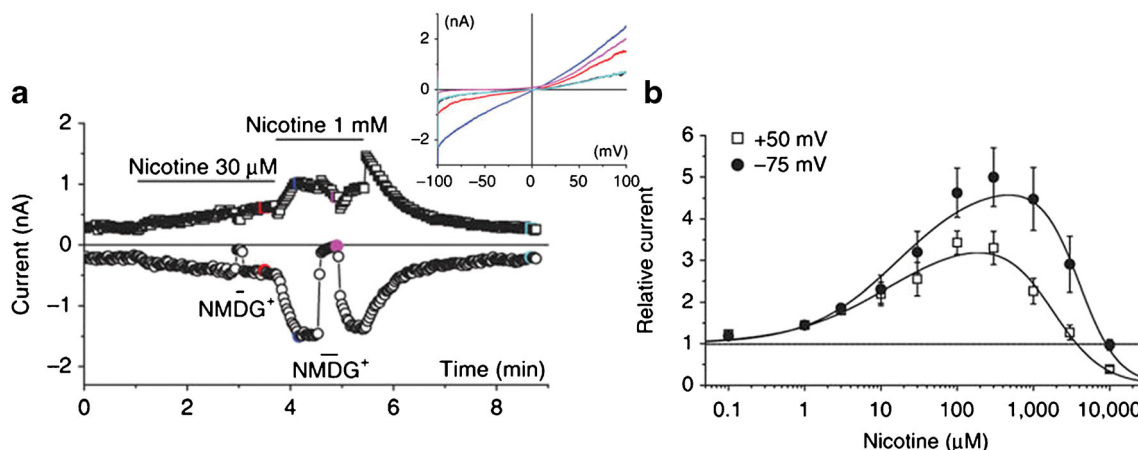


Fig. 5 Activation of mTRPA1 by nicotine. **a** Time course of the effects of nicotine on the amplitude of mTRPA1 currents measured at -75 and $+50$ mV in stably transfected CHO cells. The horizontal lines indicate the periods of extracellular application of nicotine at the indicated

concentrations. The colored data points correspond to the current traces shown to the inset. **b** Concentration-dependent modulation of mTRPA1 currents by nicotine. Reproduced with permission from Talavera et al. [206]

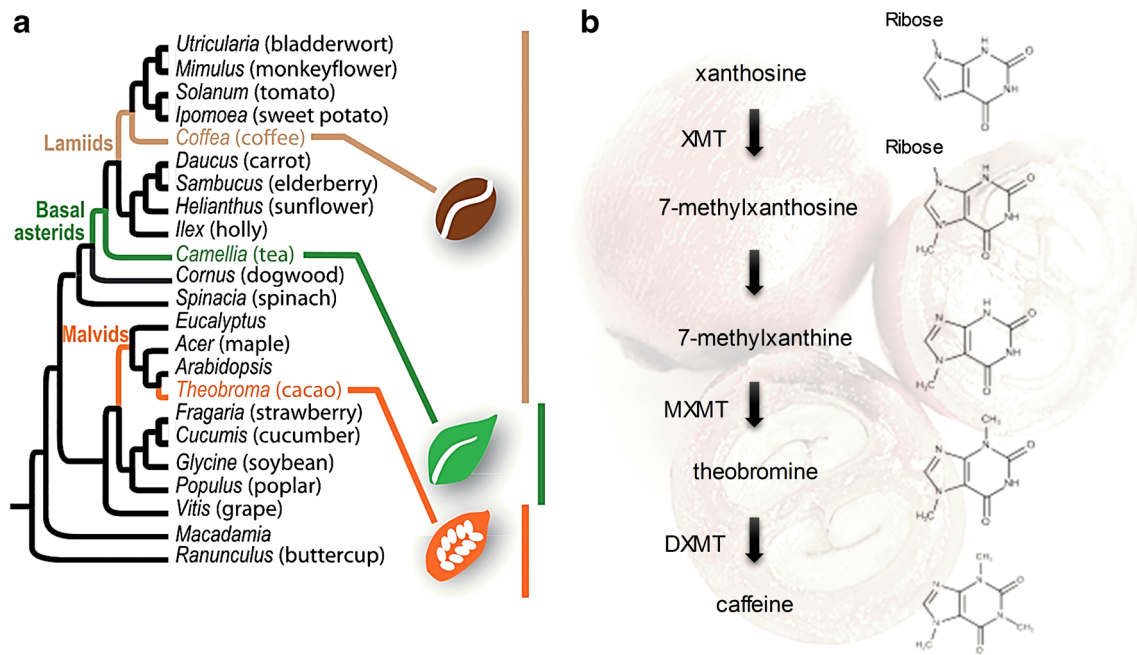


Fig. 6 Caffeine-producing plants and its biosynthesis. **a** Phylogeny tree of the evolutionary position of caffeine-producing plants with respect to other eudicots. Caffeine is commonly produced by plants belonging to *Coffea* and *Rubia* genus and many other plants such as *Theobroma* (cacao), *Ilex* (yerba mate), *Paullinia* (guaraná), *Cola* (cola nut), and *Camellia* tea (tea) species, although they evolved distinct pathways to produce this chemical [47, 53]. *Coffea* plants are hard wood trees or shrubs producing a fruit, containing usually two seeds called coffee beans [47]. Caffeine is present in the highest concentrations in the young shoots

and leaves. Depending on species green coffee beans contain variable amounts of caffeine (up to 3.3% of dry mass) [14, 15]. **b** Simplified schema of the caffeine biosynthetic pathway involving three methylations of nucleotide xanthosine. Three methyltransferase enzymes are essential in the synthesis: xanthosine methyltransferase (XMT), theobromine synthase [7-methylxanthine methyltransferase (MXMT)], and caffeine synthase [3,7-dimethylxanthine methyltransferase (DXMT)]. Reproduced with permission from Denoed et al. [53]

also called the oxidative burst [6, 56, 115, 169], is induced by activation of a membrane-bound NADPH oxidase [54, 103].

ROS plays several function during pathogen infection, being direct antimicrobial agents, activator of several defense genes,

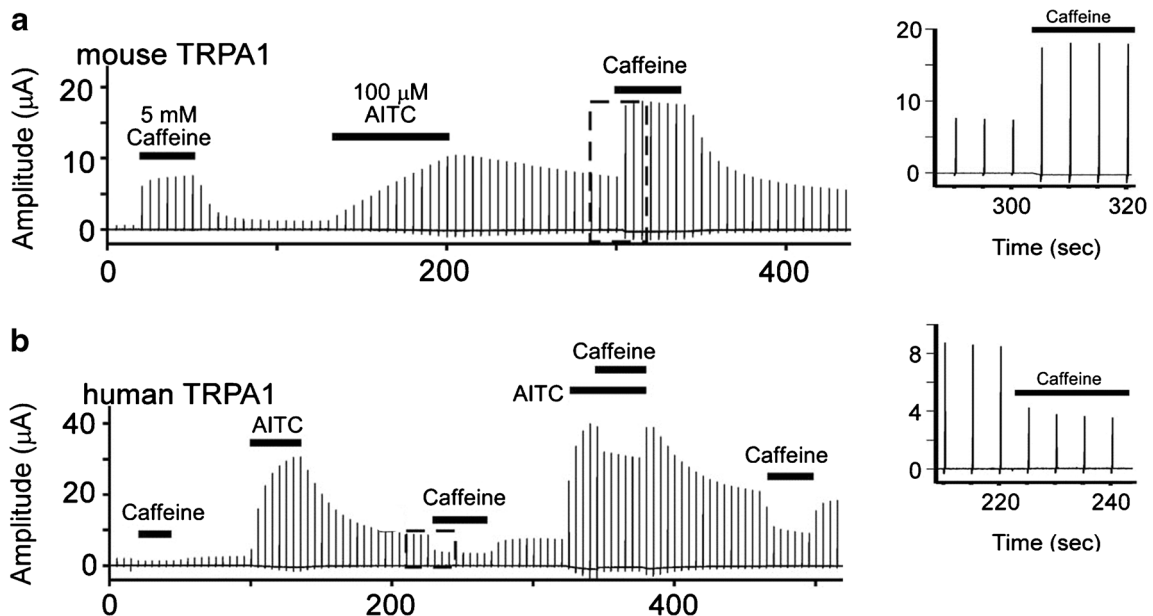


Fig. 7 Differential effect of TRPA1 activation by caffeine. **a**, **b** Current recordings using two-electrode voltage clamp (−100 to +100 mV) from *Xenopus* oocytes expressing mouse TRPA1 (**a**) or human TRPA1 (**b**). Agonist application is indicated by the bars. Right panels represent an

expanded view of the boxed region in **a** and **b**. Reproduced with permission from Nagatomo and Kubo [150] “Copyright (2008) National Academy of Sciences, U.S.A.”

inducer of the hypersensitive response (HR), cell death, salicylic acid production, and systemic acquired resistance (SAR) [56, 115]. ROS can be sensed by multiple TRP channels in sensory neurons, including TRPA1, TRPV1, TRPV4, TRPC3–5, TRPM2, TRPM7, and TRPM8 [7, 142, 152, 153, 160, 191, 216].

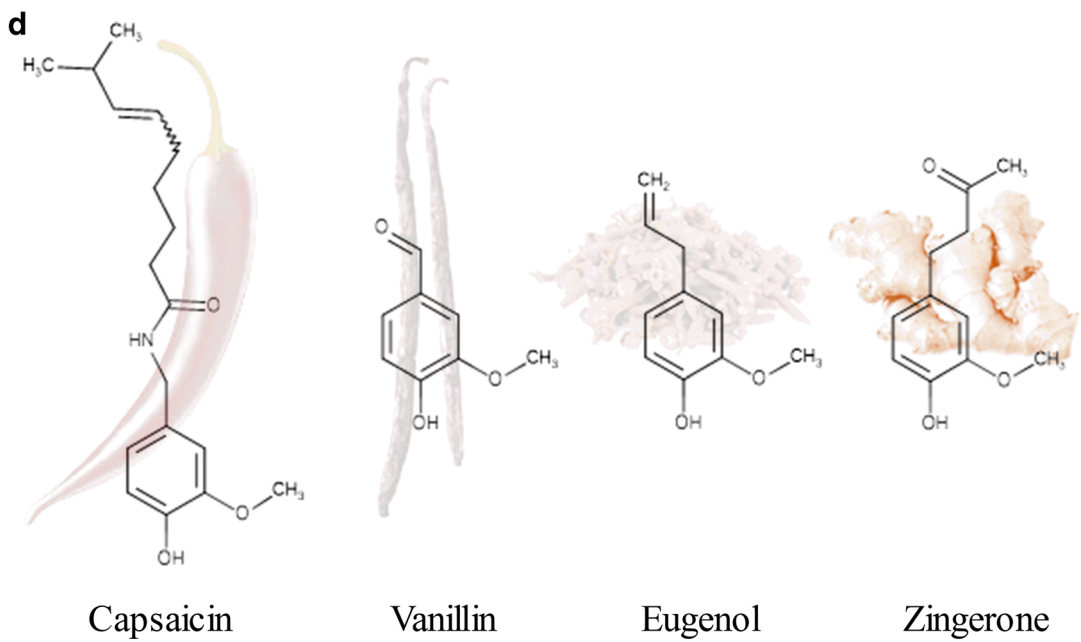
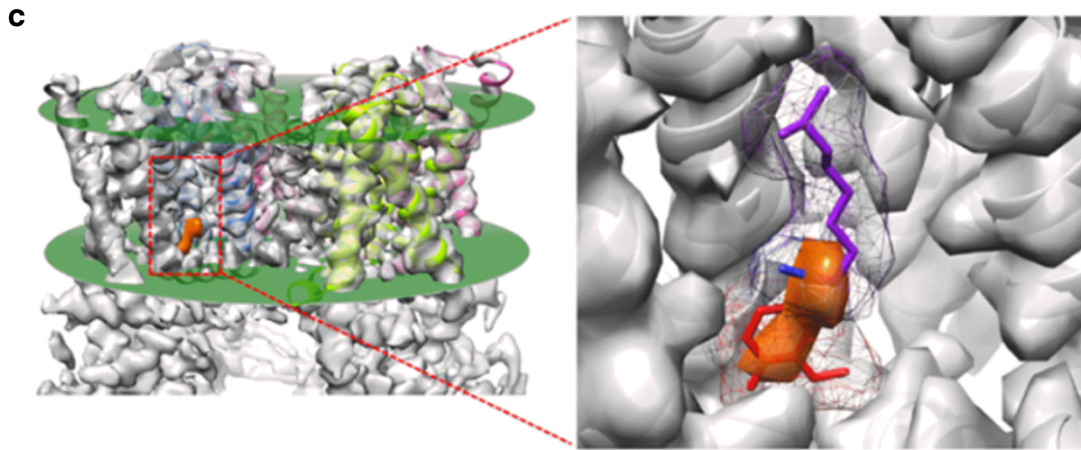
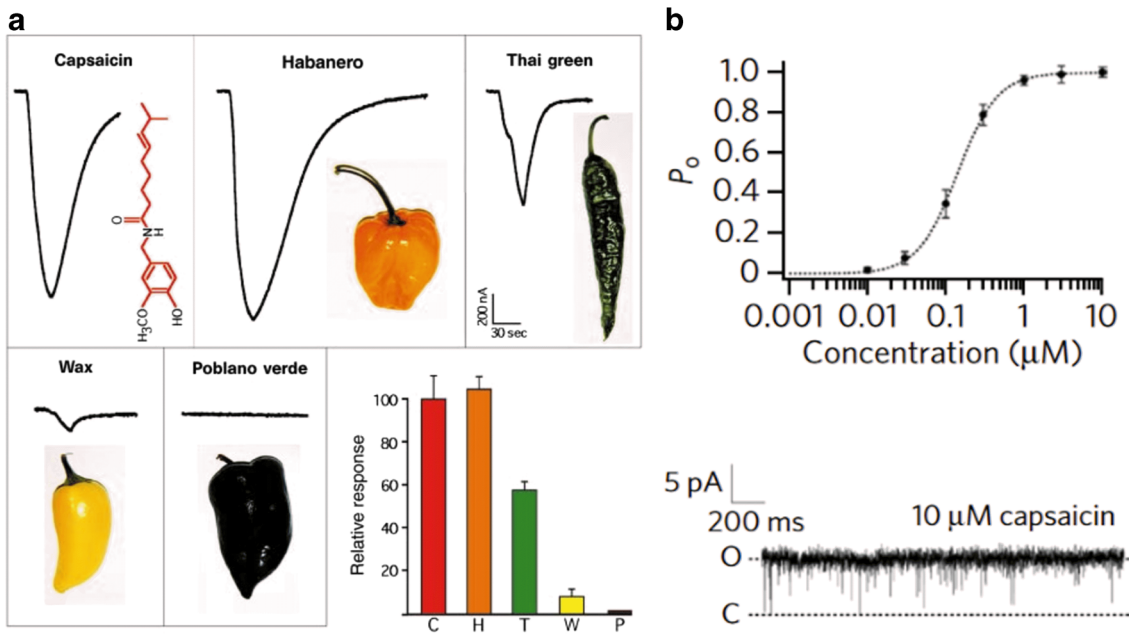
Indisputably, TRPA1 acts as a one of the major sensors of ROS and reactive nitrogen species (RNS), including nitric oxide (NO) and peroxynitrite (ONOO⁻) [144, 205, 208]. It is known that these chemicals produce covalent S-nitrosylation of TRPA1, but the full mechanism of activation by reactive species is still unclear. It is possible that channel is directly gated by these compounds [8] or an indirect activation mechanism could be involved induced by for instance generation of lipid peroxidation products [202]. TRPA1 activation is also essential in responses to hypoxic conditions, resulting in cardiovascular and respiratory regulatory reflexes [111]. In *Trpa1* KO mice, VG neurons responses to hyperoxia and mild hypoxia were abolished. TRPA1 may be activated by hypoxia via the reduction of proline hydroxylase activity, leading to decreased hydroxylation of P394 within the channel ankyrin repeats relieving inhibition. On the other hand, hyperoxia induces channels C633 and C856 oxidation [204]. Taken together, the recent literature on the interactions between reactive molecules and TRPA1 or other sensory TRP channels suggest their roles as alarm systems protecting herbivores from harmful plant defenses.

Vanilloids

Belonging to the plant genus *Capsicum* (Greek *kapto*, “to bite”), chili peppers are well-known for their pungency. Fruits of the plants from this genus contain the chemical capsaicin (8-methyl-N-vanillyl-6-nonenamide) and other structurally related compounds, called capsaicinoids, such as dihydrocapsaicin, nordihydrocapsaicin, homodihydrocapsaicin, and homocapsaicin [147]. Capsaicin belongs to the vanilloid family, which also includes vanillin (vanilla bean), eugenol (bay leaves or cloves oil), and zingerone (ginger) (Fig. 8d). Capsaicin is highly lipophilic, which explains the lack of pungency alleviation by drinking water after oral intake. The main function of these chemicals is to discourage consumption by plant herbivores, but on the other hand, the primary function of the plant fruit should be facilitation of the seed distribution. Without doubt, *Capsicum* plants evolved a specific strategy through which mammals are repelled, but birds are not and serve as main seed distributors [210]. It has been observed that consumption by birds is advantageous to these plants, as these animals excrete the seeds in places convenient for germination, such as under other plants, assuring shade beneficial for seed survival and shelter from consumers [209, 210]. Interestingly, seeds excreted by birds are able to germinate, but no germination occurs after ingestion by mice. This was attributed to the distinct ways in

Fig. 8 TRPV1 activation by capsaicin. **a** Effect of peppers extracts with different pungencies on currents recorded in a *Xenopus laevis* oocyte expressing TRPV1 channels. Reproduced with permission from Caterina et al. [35]. **b** Concentration-response curve (top) and single channel recordings (bottom) of TRPV1 upon application of capsaicin. **c** Localization of the binding site of capsaicin (orange) in the TRPV1 structure. Reproduced with permission from Yang et al. [232]. **d** Chemical structures of capsaicin and structurally related vanillin, eugenol and zingerone.

which the seeds are ingested by those animals. In birds, seeds are not chewed on, pass the gut very quickly and come out intact. In contrast rodents and other mammals damage the seeds by chewing and by the effects of acidic juices during food digestion. For example, it was found that seeds of *Capsicum chacoense* (native to Bolivia, Argentina, and Paraguay) that pass through the digestive system of the small-billed Elaenia, a bird of the family *Tyrannidae* had less pathogens (*Fusarium* fungi) and were devoid of volatile chemicals that attract granivorous ants. This resulted in a 370% rise in seed survival rate [67]. Reduction in fungi load on *C. chacoense* seeds is extremely important because it represents a major seed mortality factor [67, 211]. A key question is why mammals, but not birds, display such strong aversion towards hot peppers? The answer lays in the different sensitivity of nociceptive nerve fibers of these animals towards capsaicin, a specific agonist of TRPV1 (Fig. 8). It was shown that bird TRPV1 orthologs lack sensitivity to capsaicin and other vanilloids [93]. Comparison of mammalian and different avian TRPV1 sequences revealed only 68% amino acid sequence conservation [93]. The capsaicin-TRPV1 interactions have been studied extensively by creation of mammalian channels chimeras with vanilloid-insensitive TRPV2 or avian or rabbit capsaicin-insensitive TRPV1. These studies identified essential residues in the TM2 and TM3 necessary for capsaicin binding with a single, evolutionary conserved amino acid (Y511), crucial for vanilloid sensitivity [93]. The sensitivity differences between species could be attributed to I550 residue as substitution of this amino acid to threonine rendered rabbit TRPV1 vanilloid sensitive [72]. Moreover, the inverse substitution, T550I, produced reduction of capsaicin responses in human and rat TRPV1 [72]. Taken together, these results suggested that the hydrophobic tail of capsaicin interacts with aromatic Y511 residue and that several polar amino acids including M547, S512, and R491 are important in accommodating its vanilloid moiety [72, 93]. Multiple molecular dynamics simulations showed “tail-up, head-down” configurations of capsaicin in the vanilloid pocket (Fig. 8c), where the aliphatic “tail” forms van der Waals interaction with the channel and contributes to binding affinity. The vanillyl “head” and amide “neck” of the molecule interact with the channel via hydrogen bonds, which grant specificity for the capsaicin binding [46, 75, 119, 162]. The cryo-EM confirmed the localization of the vanilloid binding pocket, where interactions occur between the vanillyl group of capsaicin and Y511, while the adjacent S512 also participates via hydrogen bonds [33, 119]. Moreover, depending on the



presence of vanilloids, Y511 assumes two distinct rotamers, suggesting an “induced fit” mechanism [33]. TRPV1 opening, upon capsaicin binding, is associated with major structural rearrangements of the pore helix and selectivity filter, in addition to distinct dilation of a hydrophobic constriction at the lower gate [33]. Therefore, capsaicin sensitivity that greatly varies between animals has important implications for understanding the complexity of the interactions between plant survival strategies and plant consumers. For birds, lack of sensitivity to capsaicin is beneficial as they can forge on this food source. On the other hand, the high sensitivity of other species, in particular mammals, acts and allows plants to use capsaicin as a strong repellent. Overall, these mechanisms are highly beneficial for the plants, as they ensure that their seeds are spread by the optimal vector and protect the plant from overconsumption.

Vanillin, a volatile chemical structurally similar to capsaicin, is produced by approximately 110 species of the vanilla plants, including *Vanilla planifolia*, *Vanilla tahitensis*, and *Vanilla pompon* [69]. This primary component of the vanilla bean extract is largely used in foods, beverages, cosmetics, and pharmaceutical industries as a flavoring agent. Vanillin has antimicrobial activity [129] and is a biologically active attractant of orchid bees [62]. Together with vanillin, more than 40 other chemicals such as 8-cineole, eugenol, or linalool are present in bees attracting extracts [62]. Vanillin has mild CNS effects and is often regarded as an aphrodisiac. Although, vanillin acts mainly on the olfactory system, this compound was shown to act also on TG neurons. In mammals, this odorant was shown to activate TRPV3 at rather high concentrations (above 10 mM) [228]. Later, it was shown to activate trigeminal TRPA1 and TRPV1 and inhibit TASK1 channels [130]. Since TG were activated with lower vanillin concentrations (1 mM) and TRPV3 expression in trigeminal neurons is arguable, TRPA1 and TRPV1 activation was linked with the observed activity.

Eugenol is a volatile chemical produced by various plants, where it functions as a floral attractant for pollinators as well as an antimicrobial agent [107]. Eugenol and its derivatives are present in essential clove and clocimum oils obtained from *Eugenia carophyllata* and *Ocimum gratissimum*, nutmeg, cinnamon, basil, and pimento berry [102]. This compound contributes the specific aroma of many fruits, as in the case of ripe strawberries [13]. Eugenol acts as repellent of beetles, inhibits egg and larvae development of the greater grain weevil *Sitophilus zeamais* [163], and can be toxic for humans, inducing liver damage and failure, respiratory distress syndrome, and CNS depression [60]. The structural similarity between eugenol and capsaicin suggests analogous molecular mechanisms of action. Indeed, it has been shown that it possesses antinociceptive properties similar to once induced by capsaicin, attributed to TRPV1 activation [228, 231]. However, eugenol-induced responses were slower and characterized by smaller amplitudes, than those to capsaicin [173, 231]. Additionally, eugenol shares many properties with local

anesthetics, such as the ability to alleviate tooth pain, which relate to the inhibitions of voltage-gated Ca^{2+} and Na^{+} channels [38, 121, 174]. Eugenol can also block production of inflammatory pain-metabolites as prostaglandins through inhibition of cyclooxygenase-2 and lipoxygenase activity [55, 213]. Other TRP channels, namely, TRPA1, TRPM8, and TRPV3 are also activated by this volatile chemical [17, 228]. Oral application of eugenol induces pungent sensations as warmth and thermal pain, which were proposed to be mediated by TRPV3 and TRPV3-mediated enhancement of thermal gating of TRPV1 expressed in lingual polymodal nociceptors [105].

Zingerone is a crucial contributor to the specific flavor of dried and cooked ginger (*Zingiber officinale*). It is produced by heat conversion of gingerol, another active ginger chemical [88]. It is structurally similar to capsaicin, suggesting that it may act on TRPV1, but has a shorter hydrophobic moiety and lacks an acyl-amide moiety. These structural differences are likely to be responsible for a more rapid onset and a faster decay of zingerone's gustatory and trigeminal responses, compared to those of capsaicin [127]. On the other hand, zingerone induces TG desensitization and tachyphylaxis, similarly to capsaicin [127]. As described above, the aromatic portion of the Y511 residue in TRPV1 is necessary for the interaction with the vanilloid moiety of capsaicin. It was proposed that zingerone and other capsaicin-related chemicals can interact only partially with Y511, resulting in weaker TRPV1 agonist activity [72].

Another target of zingerone is TRPA1, acting through a mechanism distinct from that of AITC. Instead, it was proposed that TRPA1 activation by zingerone involves intracellular Ca^{2+} mobilization from internal stores through Ca^{2+} -induced Ca^{2+} -release mechanisms [112]. Finally, different fruit fly species are distinctly attracted to plants producing zingerone, eugenol, and its derivatives, suggesting for a differential expression of the relevant chemosensory TRP channels in these species [207].

Plants from the *Piper* genus, such as black pepper and long pepper, produce another pungent vanillamide, piperine. In addition, pepper plants contain roughly 145 different chemicals, including chavicine, piperettine, piperiline, piperlonguminine, 4,5-dihydropiperlongumine, pellitorine, pipericide, guineensine, and sylvatine, all contributing to the characteristic spicy, tingling, and warming sensations induced by black pepper [48]. These compounds are all deterrent to many animals. In nature, these chemicals act as insecticides, exhibiting low levels of threat to the environment or to human health. In fact, pepper is broadly used as flavoring agent having many beneficial effects as an antioxidant, anti-inflammatory, and antimicrobial agent [141]. Although at first glance piperine seems to be tasteless, this impression is quickly replaced by a burning sensation attributed to activation of TRPV1 [140]. Another member of the TRP superfamily, TRPA1, is also activated by pepper chemicals including

piperine, isopiperine, isochavicine, piperanine, piperolein A, piperolein B, and N-tetra, although at concentrations higher than those required to activate TRPV1 [166]. Therefore, the pungent flavor of pepper may be attributed to the activation of both channels.

Garlic, onion, and mustard: organosulfur-based defense systems

Plants from the *Brassica* genus, also known as mustard plants (mustard, broccoli, Brussels sprouts, watercress, wasabi, and cauliflower), produce the highly pungent compound allyl isothiocyanate (AITC) upon disruption of the plant tissue by pathogens or herbivores. Isothiocyanates (ITC), and several other compounds such as nitriles, thiocyanates, oxazolidine-2-thiones, and epithionitriles, are produced from glucosinolates that are hydrolyzed by the enzyme myrosinase [175]. These volatile compounds reduce oviposition and feeding activities of insects, and their strong pungency induces aversion behavior in other herbivores [68]. The pungent sensation that AITC produces in mammals was initially attributed to a specific activation of TRPA1 expressed in nociceptive neurons [20]. ITCs are highly electrophilic chemicals and can form conjugates with accessible thiol groups in proteins. In the case of human TRPA1, these were reported to be cysteines at positions C619, C639, and C663 and to a lesser extent lysine 708, all located in the cytoplasmic N-terminal tail of the channel between the last ankyrin repeat and the first transmembrane segment [80]. It was also shown that immunoprecipitated mouse TRPA1 can be covalently modified by electrophilic agonists and at least 14 other cysteine residues can contribute to channels activation [131]. The key cysteine residues of mouse TRPA1 (C415, C422, and C622) are close and within the ankyrin repeats presenting intriguing differences between two species [131]. Moreover, cysteines located in the mouse TRPA1 N-terminal can form disulfide bridges that stabilize channel structure [236]. Actually, residues can be shared to form bridges as for example in mouse TRPA1 C666–C622, C666–C463, C666–C193, and C622–C609, suggesting that channel activation may involve different N-terminal conformations [220].

Our later studies show that some TRPA1 electrophilic agonists such as AITC and cinnamaldehyde activate the channel at low concentrations but also have an inhibitory effect at high, but pharmacologically relevant, concentrations [5, 61]. In addition, although TRPA1 is a main target of ITCs, it has been demonstrated that *trpa1* knockout mice display residual aversive behavior against AITC [113]. More detailed investigations revealed that AITC also activates TRPV1 and that this channel contributes to the nociceptive effects of AITC [61, 73, 164].

In insects, AITC exposure induces mortality, adult and immature malformation, repellency, and altered development [66, 85, 189]. The mechanisms of AITC action in insects are still unresolved. However, it may involve disrupting the

activity of the mitochondrial complex IV and complex I, leading to tissue dysfunction [136, 233]. Insect feeding is also inhibited by ITC and related chemicals via a mechanism involving TRPA1 expressed in GRNs in the insects mouthparts [4, 97]. This further points to evolutionarily conserved molecular pathways involving sensory TRP channels that are implicated in chemical nociception between different organisms. Detection of harmful, electrophilic chemicals by TRPA1 emerged over 500 million years ago in a common vertebrate/invertebrate ancestor [97]. Interestingly, sensing noxious chemicals was a “secondary” function as different TRPA clades express highly temperature-sensitive channels, indicating thermosensitivity to be ancestral. In addition, the channel N-terminal region, which is highly variable in the sequence between different animals, even within same class as insects, indicates specialization of the channel properties [74, 97]. For instance, different mosquito species, express two TRPA1 isoforms (TRPA1(A) and TRPA1(B)) that not only vary in thermal sensitivity but also differently respond to electrophilic agonists [97]. This conserved, functional diversity of TRPA1 between different host-feeding species provides a clue for how this insects sense and select their host.

Furthermore, extracts from *Brassica* plants induce genotoxicity in bacteria, in fungi, as well as in mammalian cells. Different chemical substitutions of ITC have variations in their DNA-damaging capacities, as for instance, benzyl-ITC has much more effect than AITC or phenethyl-ITC [99].

Garlic (*Allium sativum*) has also adapted to deter foragers by inducing burning, irritating sensation in the mouth when cloves are consumed. Sensations elicited by garlic are attributed to activation of TRPA1 and TRPV1 channels [132, 187]. It has been shown that approximately 30% of rodent TG neurons responds to garlic extract [20]. Diallyl sulfide (DAS), diallyl disulfide (DADS), and diallyl trisulfide (DATS) activate both channels (Fig. 9b), but display higher affinity for TRPA1 [20, 109, 132]. Although garlic consumption has been shown to be highly beneficial for health [10, 18, 26, 168] and garlic is used as natural remedy for many illnesses, overconsumption is greatly toxic. Garlic extracts are mutagenic, induce anemia by lysing red blood cells, and even induce death in rats (above 5 ml of garlic juice per kg) [18, 110]. Garlic components, mainly DADS, allyl propyl disulfide, and allicin, can also induce vomiting, heartburn, diarrhea, allergic contact dermatitis, asthma, hypoglycemia, flatulence, tachycardia, and insomnia [18, 32, 71, 172]. One of the pests of garlic and other *Allium* plants is *Ditylenchus dipsaci*. This bloat nematode lives between the cells of bulbs, stems, and leaves feeding on the sap. A question is then, how can this animal live and feed on garlic juices containing so many harmful chemicals. Part of the answer may lay in the TRP channel expression patterns, as most nematode parasites possess fewer genes, often lacking TRPA, TRPM, or TRPP sequences [224]. This may lead to a reduction of noxious sensory inputs

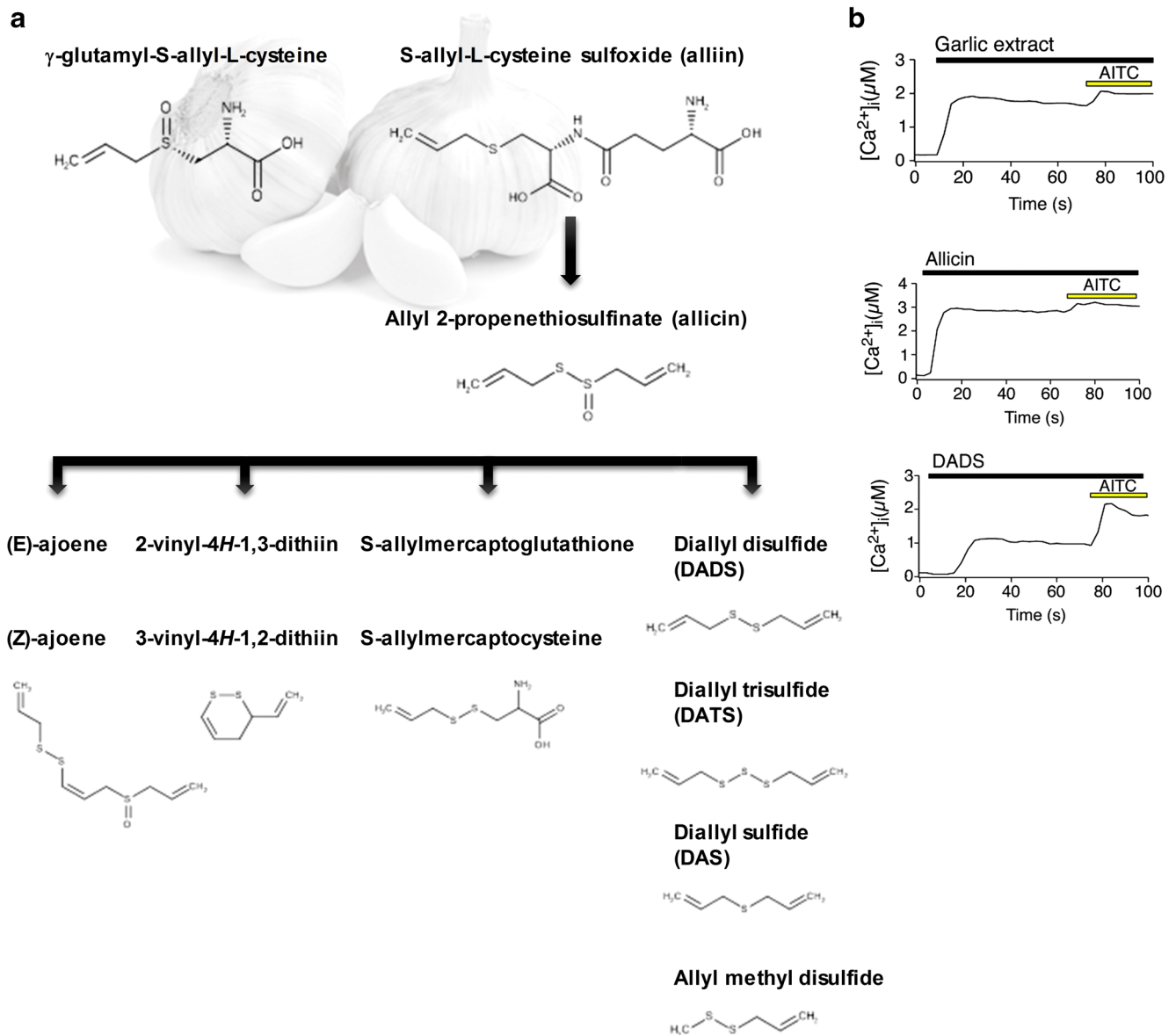


Fig. 9 Chemical structures of produced by garlic defense chemicals is associated with TRPA1 activation. **a** Two classes of organosulfur chemicals are present in whole garlic cloves: L-cysteine sulfoxides (alliin) and γ -glutamyl-L-cysteine peptides. Alliin, which accounts for about 80% of cysteine sulfoxides in garlic, is transformed to allicin by alliinase enzymes within a few seconds after clove integrity disruption [26, 219]. This short-lived chemical is further transformed into more stable sulfur compounds as DAS, DADS, and DATS [20, 26]. In fact, garlic contains more than 33 different organosulfur chemicals, as well as

many other chemicals whose functions remain unknown [138, 168, 219]. **b** Intracellular Ca^{2+} responses of rat TG primary sensory neurons expressing TRPA1 channels exposed to raw garlic extracts (1:10,000 dilution, top panel) and its derivatives 40 μM allicin (middle panel) and 200 μM DADS (bottom panel). Subsequently, all AITC-responsive neurons were also activated by garlic compounds, pointing to a role of TRPA1 in sensing these chemicals. Reproduced with permission from Bautista et al. [20] “Copyright (2005) National Academy of Sciences, U.S.A.”

recognition, which is consistent with these animals residing in environments that others would perceive as harmful.

Other plants of the *Allium* genus such as onions, shallots, chives, and leeks also produce pungent thiosulfonates and disulfides. Onion (*Allium cepa*), similarly to garlic, generates many toxic compounds causing hemolytic anemia in dogs, cats, cattle, and horses [188]. These plants contain S-alk(en)yl-cysteine sulfoxides (ACSO), whose content in different crops is regulated by genetic and environmental factors. There are six

different ASCOs produced, including S-methylcysteine sulfoxide (methiin), S-propylcysteine sulfoxide (propiin), trans-S-(1-propenyl)cysteine sulfoxide (isoalliin), S-butylcysteine sulfoxide (butiin), S-ethylcysteine sulfoxide (ethiin), and the above-mentioned S-(2-propenyl)cysteine sulfoxide (alliin) [128]. Upon cell integrity disruption by foragers, short-lived ASCOs degrade, resulting in a pungent, irritating aroma. Onion produces methiin, propiin, and isoalliin [128], resulting in the onion lachrymatory factor attributed to formation of propanthial S-oxide, 1-propenyl

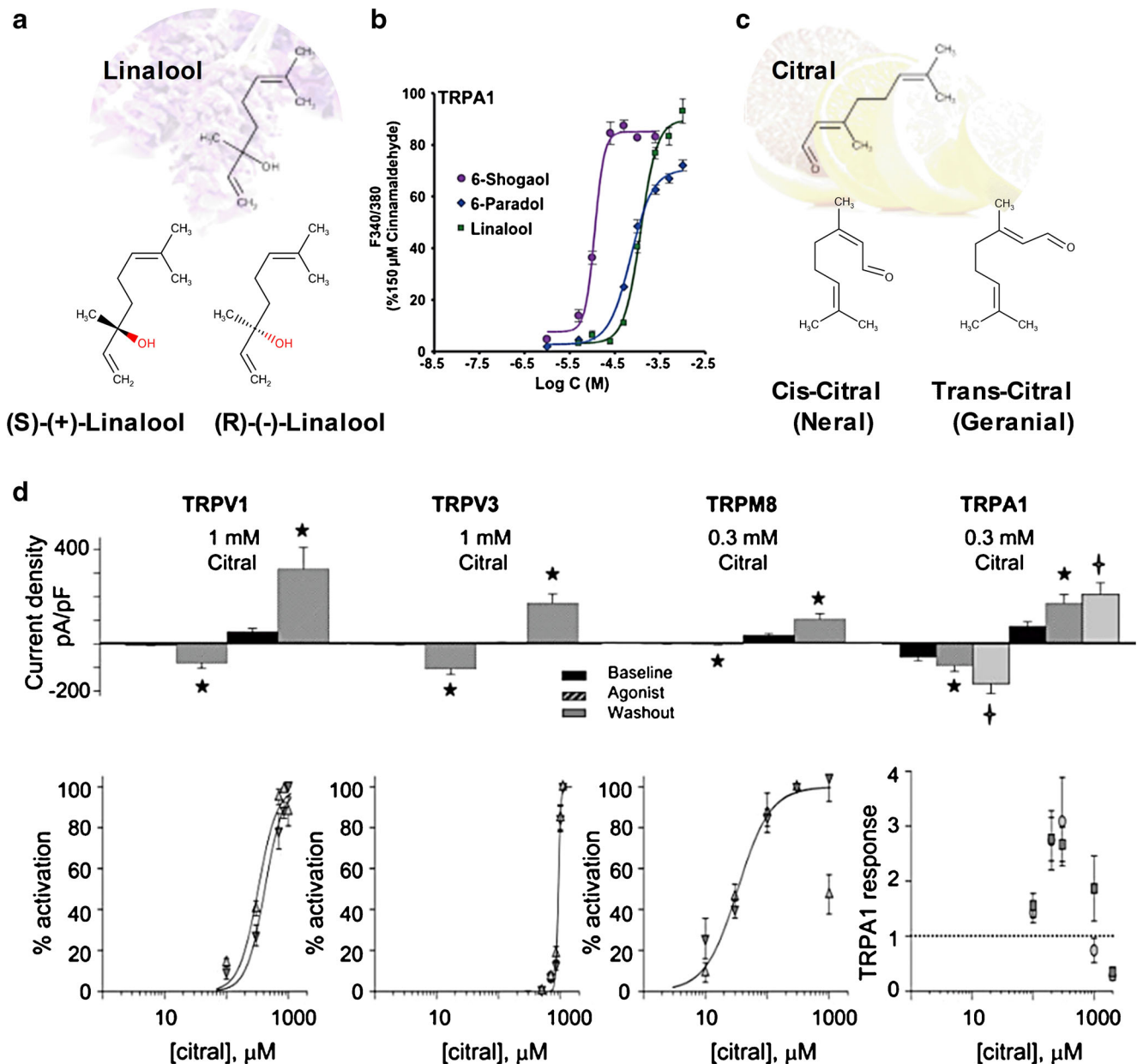


Fig. 10 Structures of linalool and citral and their activity on TRP channels. **a** Monoterpenoid alcohol, linalool, has chiral properties and is present in two enantiomeric forms; (R) and (S)-linalool. (R)-linalool is found in many other plants, such as camphor, rosewood trees, thyme, basil, lavender, and bergamot. The (S) enantiomer is found in black locust and plants from *Coriandrum* genus, palmarosa and sweet orange [24, 123, 183]. **b** Dose-response relationships for linalool, 6-shagol and 6-paradol and TRPA1 activation. **c** Chemical structures of citral and its

enantiomers. **d** Currents through the TRP channels rTRPV1, rTRPV3, rTRPM8, and rTRPA1 are increased upon application of citral. Inward currents are increased for TRPV1 by 14-fold, TRPV3 by 38-fold, TRPM8 by 2-fold, and TRPA1 3-fold (top panel). Dose-response curves (bottom panel) for citral activation of TRPV1, TRPV3, TRPM8, and TRPA1. Reproduced with permission from **b** Riera et al. [185] and **d** Stotz et al. [200]

methane thiosulfinate, and di-propyl disulfide [22, 58, 84]. In domestic animals, consumption of 5–30 g/kg of onion can result in red cell damage [188], while humans, sheep, goats, and rodents are more tolerant. All chemicals mentioned here are highly potent electrophiles, activating both insect and vertebrate TRPA1 via covalent modification. Channel opening by these compound results in avoidance, as for instance, escape behavior, due to induction of pain, coughing, and lachrymation.

Terpenoids—etheric frontiers

Terpenoids form a group of highly important plant secondary metabolites having multiple functions in primary plant physiology (photopigments, electron carriers, membrane function modifiers, etc.), as well as in the protection against abiotic and biotic stress [1, 212]. This group contains over 40,000 structurally different chemicals derived from isopentenyl diphosphate (IPP) and

its allylic isomer dimethylallyl diphosphate (DMAPP) [212]. Plants produce terpenoid precursors via two independent, genetically regulated pathways. One is the cytosolic mevalonic acid (MVA) pathway, involved in the synthesis of sesquiterpenoids, polyprenols, phytosterols, and brassinosteroids, and the second is the plastidial methylerythritol phosphate (MEP) pathway, implicated in the synthesis of hemiterpenoids, monoterpenoids, diterpenoids, carotenoids, cytokinins, gibberellins, chlorophyll, tocopherols, and plastoquinones [77, 78, 212]. Many terpenoids are highly volatile chemicals associated with plant fragrance, acting as insect attractants or repellants [190]. For instance, a monoterpenoids mix containing d-limonene, β -myrcene, and *E*- β -ocimene, released by flowering plants such as the monkeyflowers (*Mimulus*), is crucial to attract specific pollinators [28]. Plants from the orchid genus (*Satyrium*) emit fragrance mixtures composed of at least 70 different compounds, of which many are monoterpenoids, sesquiterpenoids, and benzenoids. The specific scent of these plants is necessary for attracting only pollinating *Atrichelaphinus tigrina* beetles and *Hemipepsis hilaris* wasps [91]. The most abundant in the orchid fragrance mixtures are linalool, eucalyptol, elemicin, α -pinene, myrcene, and 2,6-dimethyl-1,5(*Z*),7-octatrien-3-ol [91]. Many of these compounds, especially linalool, are able to directly induce electrophysiological responses in antennae of the pollinating beetle species, making them generalists visiting these plant species. Linalool (3,7-dimethyl-1,6-octadien-3-ol) (Fig. 10a) has a sweet, pleasant scent, occurring widely among diverse monocot and dicot plant families [106]. Remarkably, it has been shown that linalool chirality determines the specific interactions between bees and orchids [91]. Similarly, bees could respond contrarily to the linalool enantiomers, as this compound is dominant in secretion of receptive females [25]. Another theory states that the scent emitted by orchids repels other pollinating insects and animals, as absence or reduced concentration of the compound attracted bats and flies. Linalool was shown to activate members of mammalian TRP channel superfamily, TRPA1 (EC_{50} 117 μ M) (Fig. 10b) [185] and TRPM8 (EC_{50} 6.7 mM) [21]. Interestingly, in sensory trials, linalool failed to induce the burning, painful feeling associated with TRPA1 activation [185], which may be explained by its analgesic properties via interaction with other targets. Linalool enantiomers were shown to reduce peak amplitudes of fast-conducting compound action potentials of frog sciatic nerve fibers [165], to inhibit action potentials of rat dorsal root ganglion neurons [118], and to inhibit voltage-gated Na^+ channels expressed in newt olfactory receptor cells [151].

Citronella essential oil is a pale to dark yellow liquid with woody, grassy, or lemony scent, produced by the plants *Corymbia citriodora*, *Cymbopogon nardus*, *Java citronella*, and more than 50 other plants. This oil is well-known by its insect repellent properties (especially mosquito) and antimicrobial functions, and it is often used in ancient Indian and South-east Asian traditional medicines. Originally, citronella

oil was purified for use in perfumery and its repellent properties were exploited as early as the beginning of the twentieth century [134]. Nowadays, the citronella essential oil is used in cosmetic, pharmaceutical, flavor, and fragrance industry. It contains more than 80 chemicals, including as main components the monoterpenes citronellal (3,7-dimethyl-6-octenal), citronellol (3,7-dimethyloct-6-en-1-ol), citral (3,7-dimethyl-2,6-octadienal), and geraniol ((2*E*)-3,7-dimethyl-2,6-octadien-1-ol). It has been shown that aversion to citronellal in *Drosophila melanogaster* involves two distinct olfactory pathways. The first implicates insect-specific olfactory receptors and the second involves the function of *Drosophila* TRPA1(A) isoform downstream of a GPCR. Moreover, human and mosquito TRPA1 are activated by citronellal, with higher sensitivity than for dTRPA1(A). Although citronella is a well-known bug repellent, it should not be used at concentrations above 10% due to induction of severe skin reactions.

Citral (Fig. 10c), characterized by its bittersweet, lemony flavor and odor, is an active ingredient of lemongrass oil, lemon peel, citronella, and palmarosa grass and has been characterized as insect repellent [76]. Citral, in nature found as two more stable isomers, *E*-citral (neral) and *Z*-citral (geranial) [178], acts via GPCRs in olfactory epithelia, but was also shown to be an agonist of TRPV3 and partial agonist of TRPM8, TRPV1, and TRPA1 (Fig. 10d) [200]. Citral is an α,β -unsaturated aldehyde, so it may well activate TRP channels by electrophilic modification of cysteine and lysine residues. As mentioned above, detection of aversive electrophiles is one of the ancestral defense mechanisms conserved in invertebrates and vertebrates. Furthermore, citral was shown to interact with the transmembrane segments TM2-TM4 of TRP channels [200], which contain residues crucial for TRPV1 activation by capsaicin and TRPM8 activation by menthol [16, 87, 217]. However, a capsaicin-insensitive TRPV1 point mutant at residue Tyr 511 is activated by citral, suggesting that the binding sites for these two compounds are different [200].

Conclusions

Plants produce many aversive compounds serving to protect themselves from herbivores. This urges plant consumers to evolve specific mechanisms of chemicals recognition by specialized receptors. The analysis of the feeding ecology of different organisms demonstrates that aversive behaviors play crucial roles in the fitness of herbivores. TRP channels are one group of receptors specialized in detecting harmful stimuli and triggering protective responses in organisms. Whereas much progress has been made in the last decade in the understanding of the structure and functions of TRP channels, the evolutionary link between bypassing plant defense mechanisms and TRP channel functional expression in the animal kingdom is largely unexplored. Summarizing, in this review,

we illustrate the involvement of TRP channels in detection of harmful stimuli and the evolutionary adaptation of organisms consuming plants that use TRP channel agonists as defense mechanisms. Since the expression pattern and ligand sensitivity of TRP channels varies between species, this presents an intriguing evolutionary adaptation to their specific habitat and life cycles.

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