

New Insights into the Roles of cAMP and InsP3 on Odor-Processing and Olfactory-Driven Behaviors

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Short-Communication

The olfactory system is one of our fundamental senses, responsible for the detection of airborne chemicals from our surroundings. These chemicals include small volatile molecules, proteins, peptides to gaseous substances, such as carbon dioxide, and provide valuable information regarding the location of food, mates and potential danger. The ability of the olfactory system to detect and orchestrate appropriate behavioral response to these chemicals is critical for the organism's survival. Given the vast array of chemical substances in the environment, it seems inevitable that the olfactory system uses a large repertoire of odorant receptors (ORs), signaling pathways and olfactory subsystems to sample its surroundings.

Over recent decades, the molecular and cellular mechanism underlying odor signal transduction has been studied in great detail and is well established. We now know that in vertebrates and *C. Elegans*, odorants interact primarily with ORs, which are G-protein coupled receptor (GPCRs) located on the membrane of olfactory receptor neurons (ORNs). Binding of an odor to an OR triggers the activation of second messenger cascades, of which, adenosine 3', 5'-cyclic monophosphate (cAMP) was one of the first second messengers proposed to mediate olfactory transduction at ORNs. Experiments using biochemical and molecular biological techniques provided evidence that when an odor binds to an OR, it stimulates the rapid synthesis of cAMP via the activation of GPCRs and adenylyl cyclase [1-2]. Increased levels of cAMP cause the opening of cyclic-nucleotide-gated channels (CNGC) [3-4], which are highly permeable to Ca²⁺ [5] and their opening enhances Ca²⁺ concentration within the ORNs [6-7]. cAMP-mediated Ca²⁺ increase has been shown to activate a second conductance, such as Cl⁻ or K⁺ [8-9], which ultimately results in the generation of action potentials that carry odor-information to the first-order olfactory centre (e.g. olfactory bulb) in the brain.

Studies carried out in the last decade also indicate that cAMP is not the only second messenger mediating olfactory transduction at ORNs. There is considerable evidence indicating that some odorants do not induce an increase in cAMP but rather the concentration of inositol, 1, 4, 5-triphosphate (InsP3) is increased [1,10]. InsP3 formation can increase intracellular Ca²⁺ [11] and activate Ca²⁺-dependent K⁺ channels or Ca²⁺-dependent non-specific cation channels [1,12-13], which in turn generate receptor potential and depolarize the olfactory neuron, thus providing a signal for further neuronal processing. In addition to cAMP and InsP3, diverse olfactory transduction pathways, such as the activation of cAMP/cGMP-gated channels [14] or the generation of receptor potentials via activation or blockage of K⁺-channels [15], have been reported to mediate olfactory transduction at ORNs.

Unlike in vertebrates and *C.elegans*, in insects, the mechanism underlying odor signal transduction remains somewhat controversial. In insects, both metabotropic [16-18] and inotropic [19-20] mode of signaling have been proposed to mediate olfactory transduction at ORNs. Until recently, insect ORs were considered to be a member of GPCRs [21-22]; however, new evidence suggests that insect ORs exhibit a different membrane topology and differ in OR protein sequence from vertebrate ORs [23-24]. Most insect ORs consist of a heteromeric complex and express two distinct receptors: a universal co-receptor Or83b, and one of the common ORs [25]. Insect ORs have been shown to function both as odorant receptors and as ion channels [20]; nevertheless, there are conflicting reports on whether the ionotropic insect ORs use intracellular messengers during odor transduction processes. For instance, a study by Sato et al. [19] reported a fast ionotropic response and found no evidence of the involvement of G-protein or cAMP, cGMP or InsP3 while Wicher et al. [20] showed that insect ORs induce the synthesis of cAMP through a G-protein that in turn activates Or83b, which serves as a cAMP-gated ion channel.

It is clear that there are fundamental differences in odor-transduction machinery between vertebrates and insects; however, despite these differences, the odor-coding strategy remains similar between these two groups. In both vertebrates and insects, odorants are encoded by the activation of spatially distinct neural ensembles, which respond with characteristic temporal dynamics. At the level of ORNs, it has been shown that odorants belonging to one chemical family are detected by ORNs expressing the same OR. The expression of ORs follows the one receptor-one neuron rule according to which, a given ORN expresses only one receptor gene [26-28]. ORNs expressing the same OR extend axons that converge on the same glomerulus in the primary olfactory center in the brain [29-31] and in this manner; information about each odorant is conveyed via a segregated pathway to the brain. Each odor is encoded in combinatorial fashion, and different odorants are recognized by different combinations of ORs [32-33]. At the level of antennal lobe/olfactory bulb, major neural computations occur as the ORNs make synaptic connection with projection neurons (PNs), which are the principal output neurons (analogous to mitral/tufted cells in vertebrates), and the local interneurons that provide lateral inhibition [34-35]. Odor information is then relayed via projection neurons/mitral tufted neurons to higher order brain centers for further processing [34-35].

At present, it is well established that the transduction mechanism at ORNs is mediated by cAMP and/or InsP3-signaling pathway in an odorant-dependent manner [36]; however, what is not completely understood is how these two signaling systems interact during odor-processing to affect the overall perception and adaptation to odorants. In our recent study [37], we performed experiments to disrupt cAMP

either alone or in conjunction with the InsP3-signaling pathway and examined how it affected odor-evoked neural responses, and the ability of flies to detect or adapt to an odor stimulus. Interfering-RNAi was used to selectively knockdown adenylyl cyclase gene (*rutabaga*), or phosphodiesterase gene (*dunce*), and InsP3-signaling pathway cellular targets, such as InsP3 receptor gene (e.g. *itpr*) or ryanodine receptor gene (*Ryr*), at ORNs axon terminal at antennal lobe. Next, using in-vivo bioluminescence Ca^{2+} imaging technique, the dynamics of ORNs axon terminal response to three different odorants (e.g. spearmint, citronella, octanol) was monitored. This was followed by olfactory behavioral assay to study how perturbation of cAMP with or without InsP3 affects perception (i.e. response that occurs after first odor-exposure) and adaptation (i.e. response to repetitive odor-exposure) behaviors. The findings of this study showed that cAMP disruption results in a smaller amplitude and shorter duration Ca^{2+} response at ORNs axon terminal at antennal lobe, an effect which is substantially amplified if both cAMP and InsP3 are disrupted. Repeated presentation of a given odorant leads to a gradual decrease in odor-evoked Ca^{2+} response in wild-type flies indicating that the ORNs of control flies adapts to an odor. Conversely, the ORNs of flies with disrupted cAMP or cAMP and InsP3 failed to adapt to an odor.

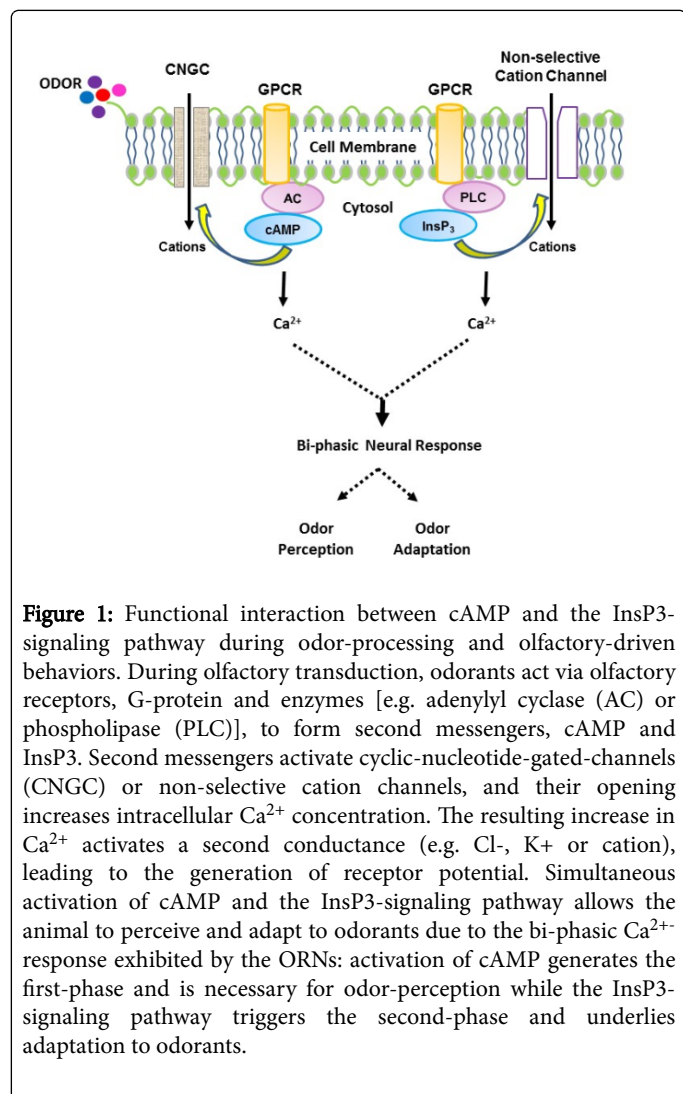


Figure 1: Functional interaction between cAMP and the InsP3-signaling pathway during odor-processing and olfactory-driven behaviors. During olfactory transduction, odorants act via olfactory receptors, G-protein and enzymes [e.g. adenylyl cyclase (AC) or phospholipase (PLC)], to form second messengers, cAMP and InsP3. Second messengers activate cyclic-nucleotide-gated-channels (CNGC) or non-selective cation channels, and their opening increases intracellular Ca^{2+} concentration. The resulting increase in Ca^{2+} activates a second conductance (e.g. Cl^- , K^+ or cation), leading to the generation of receptor potential. Simultaneous activation of cAMP and the InsP3-signaling pathway allows the animal to perceive and adapt to odorants due to the bi-phasic Ca^{2+} response exhibited by the ORNs: activation of cAMP generates the first-phase and is necessary for odor-perception while the InsP3-signaling pathway triggers the second-phase and underlies adaptation to odorants.

We had very few groups, however, which showed some degree of adaptation, indicating that odor-adaptation impairments occur in an odor and genotype-specific manner. In behavioral assay, the wild-type flies were repelled by all three odorants, and following pre-exposure, they displayed reduced repulsion to odorants mainly due to adaptation. In contrary, flies that had defective cAMP or cAMP and InsP3 were unable to correctly detect or adapt to an odor stimulus. In summary, in this study, we were able to directly assess the strength and duration of ORNs axon terminal response to odorants while manipulating cAMP and InsP3 in an intact, living fly and establish a direct relationship between ORNs axon terminal calcium response and odor-perception and odor-adaptation behaviors.

The above described study has provided new insights into the field of olfactory transduction in insects. Firstly, it has provided in-vivo functional evidence that cAMP and the InsP3-signaling pathway act in synergy to mediate olfactory perception and adaptation mechanisms in drosophila. Second, it has demonstrated the exact roles of these two signaling pathways in generating odor-evoked neural response at ORNs axon terminal at antennal lobe, and on odor-perception and odor-adaptation behaviors. For instance, a model that was proposed in the study by Murmu & Martin [37] suggested that odorants generate a bi-phasic Ca^{2+} response at ORNs axon terminal, in which cAMP plays an important role in the generation of first-phase response, while the second-phase is mediated by the InsP3-signaling pathway. These conclusions were drawn by combining the results of that study and a previous study [38], where it was shown that under control conditions, odorants generate a bi-phasic Ca^{2+} response. Disruption of the InsP3-signaling pathway completely abolished the second-phase response, whereas the first-phase remained unaffected. At the behavioral level, exposing naive control flies to an odor caused avoidance behavior, and a similar response was found in InsP3-disrupted flies; however, following pre-exposure, control flies showed odor-adaptation while the InsP3-disrupted flies were unable to adapt to an odor [38]. These results suggest that InsP3 disruption mainly impairs odor-adaptation and not the odor-perception process [38]. Interestingly, in our recent study [37], we found that unlike InsP3, cAMP disruption does affect the first-phase response, and impairs the fly's ability to detect/perceive an odor. Based on these results, we proposed that cAMP modulates the first-phase of the bi-phasic Ca^{2+} response that is observed at ORNs axon terminal and is important for olfactory perception, while the InsP3-signaling pathway modulates the second-phase and is important for odor-adaptation behaviors (Figure 1).

In view of the current controversy regarding whether or not second messengers are involved in odor signal transduction in insects, the findings of the above described study seems particularly important since it provides in-vivo functional evidence that cAMP and the InsP3-signaling pathway are involved in olfactory processing in drosophila. Our recent study strongly suggests that odorants simultaneously activate cAMP and the InsP3-signaling pathway. Activation of cAMP and InsP3 do not simply serve the role of faithfully transducing information about an odor stimulus; these two signaling systems interact with each other to influence odor-coding, and olfactory-driven behaviors.

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