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Effect of Dopamine on honey bee Central olfactory neurons

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ABSTACT

Bumble bees live inexpansive provinces of thousands of people, which require compelling correspondence to guarantee efficient working of the settlement overall. Pheromones, synthetic concoctions utilized for this reason, assume an essential job in numerous aspects of the bumble bee life. Guarded sessions are no special case, and bumble bees utilizepotent alarm pheromones to caution their nestmates of the presence of huge (generally mammalian) gatecrashers. In anycase, pheromones are not by anymeans the only odorants that are critical to honeybees. These notable pollinators like wise depend on their feeling of smell to discover and recognize compensating blooms when they go out foraging, and the nectarthey bring home is frequently scented. Along theselines, botanical smells areas central to the science of bumble bees as pheromones. In such cases communications frequently happen that can alter or even smother the bug's response to its pheromone. The experiment was carried out to explore if and how common plant scents ordinarily encountered by honey bees amid foraging regulate aggression, specifically when they are joined with the alarm pheromone. Bumble bees (Apis mellifera) are bugs living in states with a perplexingsocial association. Their home contains sustenance stores as nectar and dust just as the brood, the ruler and the honey bees themselves. These assets must be defended from a wide range of predators and parasites, an errand that is performed by laborers, called protect honey bees. Gate keeper stune their response to both the idea of the risk and the ecologicalconditions, so as to accomplish an efficient exchange off among safeguard and loss of foraging workforce. By releasing alarm pheromones, theycan select different honey bees toenable them to ealwith huge predators. These synthetic concoctions trigger both rapid and longer-term changes in the conduct of adjacent honey bees, hence preparing them for barrier. Here, we survey our current comprehension on how this arrangement of occasions is performed and regulated relying upon an assortment of components that are both extrinsic and intrinsic to the state. The present study shows the neural bases of bumble bee aggression and feature inquires about avenues for future examinations here. This investigation is the first to uncover the modulatory activities of DA on ionic flows in bumble bee AL neurons. It also demonstrates that a critical level of AL neurons are touchy to DA and that DA diminishes the adequacy of Ca2+-actuated K+flows in these cells.

Keywords: olfactory, charybdotoxin, dopamine, Apis cerena, Apis florea, Apis dorsata, etc.

INTRODUCTION:

Essential olfactory focuses (antennal projections, ALs) of the bumble bee cerebrum are attacked by dopamine (DA)-immuno reactive neurons from the get- go being developed (pupalstage3), promptly before a time of fast development and compartmentalization of the AL neuropil. Here we looat the modulatory activities of DA on bumble bee AL neurons amid this period. Voltage-clasp accountsin entire cell arrangement were utilized to decide the impacts of DA on ionic flows in AL neurons in vitro from pupal honey bees at stages 4 to 6 of the 9 phases of transformative grown-up advancement. In roughly 45 percent of the neuronstried, DA (5-50×10-5M) diminished the abundancy of outward flows in the cells. Not withstanding a gradually initiating, continued out ward current, DA diminished the plentifulness of a quickly actuating, transient outward conductance in certain cells. Both of the flows adjusted by DA could be canceled by the expulsion of Ca 2+ from the outer medium, or by treatment of cells with charybdotoxin (2×10-8M), ablocker of Ca2+-subordinate K+ flows in the cells. Ca2+ flows were notinfluenced by DA, nor were A-type K+ flows (IA). Results recommend that postponed rectifier-like current (IKV) likewise stays flawless within thesight of DA. Taken together, our information demonstrate that Ca 2+- subordinate K+flows are focuses of DA adjustment inbumble bee AL neurons. This examination loans backing to the speculation that DA assumes a job in the creating mind of the honey bee.
Amid transformation, the focal sensory system of the bumble bee, Apismellif era, experience essensational development and revamping. No place are the progressions more striking than in the essential olfactory focuses (antennal projections, ALs) of the mind. Around pupal stage 2 of the 9 phases of transformative grown-up advancement, antennal tangible afferent neuron senter the ALs. Their landing triggers the arrangement of notice able subunits of synaptic neuropil called glomeruli, which are the utilitarian subunits of the neuropil. Every glomerulus contains the terminal arbors of antennal tactile afferentneurons, procedures of neighborhood interneurons, dendrites of projection (yield) neurons, and consequences of divergent neurons that venture to the Als from different destinations sin the cerebrum.

Quickly before glomerulus arrangement (pupal stage 3), creating ALs are attacked by dopamine (DA)-immunore active procedures that ramify widely in the focal neuropil of the flaps. These procedures start from cell bodies situated in the sidelong deutocerebralsoma skin, back to every AL. Similar cells broaden forms in to the dorsal projection of the deuto cerebrum, just as to the protocerebrum and sub oesophageal ganglion. Around pupal stage 4 there is a flood in DA levels in the ALs, and quick neurite out growth obvious instage-5 AL neurons invitro is improved by introduction to DA. While the character of the receptors that intercede the impacts of DA still can't seem to be resolved, mRNAs for 3DA receptor qualities, Amdop1, Amdop2 and Amdop3 have been identified in cells that encompass the creating AL neuropil of the honey bee. The declaration of these qualities, specifically Amdop2, is unequivocally formatively managed, recommending that DA assumes a focal job in the creating cerebrum of the honey bee.

In grown-upworking drones, Dal evelsinthe cerebrum, and dimensions of dopamine receptor quality articulation change extraordinarily amid the life time of the honey bee. Intriguingly, paying littlere spectto age, DA levels in the antennal projections off oragers are higher than in theanten nalflapsof honey bees performing nursing obligations proposing hat DA in antennal projections is connected to conduct state. While it has been recommended that biogenic amines, for example, DA may impact reactionedges forerrand related improvements, generally little is thought about the systems through which this aminew or ksinthemind of the honey bee.

OLFACTORY PERCEPTION AND LEARNING IN HONEY BEES :

The bumble bee A pismelli fera has been a focal creepy crawly show in the investigation of olfactory recognition and learning for over a century, beginning with pioneer work by Karlvon Frisch. Research on olfaction in bumble bees has extraordinarily profited by the appearance of a scope of conduct and neurophysiological ideal models in the Lab. Compound particles, particularly unpredictable ones, are the vessel of significant data that ma decide a creature's inevitable survival and regenerative achievement. May be thus, the feeling of chemoreception is universally spoken to in the set of all animals (Ache and Young, 2005). The job of the olfactory frame work is to decipher the unpredictable whirlpools of particles in nature and shape them into bits of significant data that will enable the creature to settle on choice sand take partin adjusted practices. Real assignments of the olfactory framework are for example there cognizable proof

of sustenance sources, the identification of conceivable threats, (for example, fire or predators), the acknowledgment of potential mates just as permitting social associations. How the sensory system works this change from the discovery of substance particles by means of the arrangement of neural portrayal suntil the formation of percepts has been the focal point of extraordinary research particularly invertebrates (Lledoetal., 2005; Morietal., 2006; Leonand Johnson, 2009; Mandaironand Linster, 2009) and in creepy crawlies (Galizia and Menzel, 2001; Laurent, 2002; Galizia, 2008). A general finding of these examinations is that the essential tenets fundamental olfactory preparing in these diverse classes of creatures are exceptionally comparable (Hildebrand and Shepherd,1997; Ache and Young, 2005). Generally, this likeness is thought to result from developmental assembly because of comparable requirements (Eisthen, 2002).

Bumble bees are socialbugs which present a wide scopeof practices depending on olfaction both inside and outside of the state. Additionally, the investigation of olfaction is effectively manageable to the research facility, since devoted conventions have been created in which honey bees show fast and hearty scent learning capacities (Menzel,1999; Giurfa, 2007). Also, the olfactory pathway of the bumble bee cerebrum has been widely depicted (Kenyon, 1896; Mobbs, 1982; Strausfeld, 2002; Kirschneret al., 2006) and the honey bee mind is effectively open to neurophysiological investigations like electrophysiological or optical imaging chronicles (Galiziaand Menzel, 2001).

OLFACTORY RESPONSES ON HONEY BEE:

Bumble bees were portrayed as enchantment well for revelations in science by Karl Von Frisch. The European bumble bee, Apis mellif era is entrenched as a model frame work to explored if ferent principal logical inquiries at the conduct, neural and sub-atomic dimensions. The olfactory molding in honey bees is broadly utilized for research in learning and memory (Menzel and Erber 1978; Menzeletal.1993; Menzeland Muller1996; Giurfa 2007) as highlights and components of learning and memory in honey bees are observed to be like those in well evolved creatures and people. A huge piece of these outcomes has originated from studies completed utilizing the olfactory arrangement of Apis mellif era as the model frame work. In Apis mellif era the scent particles are recognized by around 60,000 olfactory receptor neurons (ORN) present in sensilla situated on the receiving wires (Esslenand Kaisling1976; Kropfet al.2014). ORNs from one side in nervate a solitary comparing antennal flap (AL), the essential olfactory focus, through the T1-4 tracts of the antennal nerve(A) (Suzuki1975; Mobbs 1982; Galizia et al.1999; Abelet al.2001; Krischneret al. 2006). In the AL of Apis mellifera, ORNs neuro transmitter on to around 800.

Projection neurons (PN) and around 4000 nearby neurons (LN) in thick spheroidal structures call glomeruli which are them orpho functional unit of the AL (Hildebrand and Shepherd 1997; Anton and Homberg 1999; Hansson and Anton 2000). PNs in Apis mellif era associated with the higher olfactory focus them mushroom bodies (MB) through 5 antenna-cerebral tracts (ACT) where they neural connection with around 1,80,000 Kenyon cells (KC) (Mobbs 1982; Abelet al. 2001; Krischneretal. 2006; Mulleretal.2002; Rossler and Brill 2013; Zwakaet al.2016). While the dendrite sof the KCs in nervate the calyces, the axons venture frontally and structure the mushroom body peduncle. In this manner, theaxons of KCs bifurcate and the branches innervate the α-projection and the β-flap. Olfactory information is gotten by the lip area and inward 50% of the basal ring.

The MB calyces. ORNs have smell and focus subordinat ereaction profiles. ORNs that express comparative receptor types innervatea solitary glomerulus (Galizia and Menzel 2000). Axons of the ORNs innervate the cortex while PNs and LNs inner vatethe center of the glomeruli (Galizziaetal,1999). In the AL, scent data from the ORNs is prepared in the glomeruli, where they bring out smell cell explicit transiently designed reactions or the spatio-fleeting smell code (Laurent 1997, Galizia and Menzel 2000). These reactions are trade mark for every sort to odorant and exceptionally reproducible (Galizziaetal.1999). To comprehend this olfactory code itis fundamental to comprehend the glomerular game plan.

Despite the fact that in creepy crawlies the glomerular number, shape and course of action changes from species to species, their size, shape and are a have been accounted for to be comparable crosswise over people of same species and sex. This makes it conceivable to make an atomical maps of glomeruli for an animal variety to consider the scent code. In many species that we area ware of, every glomerulus gets contribution from all ORNs communicating a solitary receptor type. So the check of glomerulican go about as a limitation in hunting down receptor qualities in the DNA arrangement database. Among bumble bee species, the glomerular chart book is accessible for Apis mellifera (Galizia etal.1999). Among the nine

species in the class Apis, in India, the species Apis cerena, Apis florea and Apis dorsata are available generally. A similar report on the basic contrasts in the olfactory arrange men to automatons of Apis florea and Apis mellifera is accessible. Apis floreais found to have a comparative number of glomeruli and olfactory receptor qualities contrasted with Apis mellifera.

In our investigation we described the olfactory arrangement of Apis dorsata specialistas faras life systems, physiology and conduct. We thought about the glomerularassociation ofApis dorsata to that of Apis mellifera and made a computerized char tbook of the glomeruli. We contemplated the GABA positive innervations of the AL and the extra neousneurons of the mushroom body. The idea of the antenno-cerebraltracts and the game plan of the Kenyon cells and tractswere portrayed. We demonstrate that the general plan of the cell groups of Kenyon cells in the mushroom body is helped through in the course of action of axonal strands in the peduncle and the projections shaping parallel compartments.

Bumble bee laborers (Apisdorsata) were gathered from thehives in the college grounds, cooled in a coolerat 4° candmounted in plastic cylinders utilizing sticky tape. Forevery one of the analyses requiring accounts and color fills the head was immobilized with paraffin wax. For antero grade fills from antennal nerve, the scapus ofa reception apparatuswas cut and a gem of color Dextran Biotin, 3000MW, lysine fixable (BDA 3000; Molecular Probes) or Dextran tetramethylrhodamine, 3000MW, an ioniclysine fixable (D3308; Molecular Probes) was embedded utilizing apulled glassslim. The cutrecieving wire was fixed with Vaseline to counteract drying up. The creature was kept in a wet chamber medium-term to permit transport of the color. The following day the cerebrum was analyzed out and fixed in 4% PFA for 24 hours. To imaginethe efferent tracts of the AL and the MB the finger nail skin was sliced open toun cover the mind. Subsequent to expelling trachea and organs a glass anode containing color (dextranbiotin/dextrantetramethylrhodamine) was embedded in to the area of infusion and left fora couple of moments. The cerebrumwas latersecured with the bit off in ger nail skin that was recently cut and kept for 3-4 hours before analyzation and obsession.

MATERIAL & METHODS :

Casings of bumble bee brood gathered from hivesat the Department of Zoology, UniversityofOtago, were kept for times of 1wk in a humidified hat chery at 35°C. Change able grown-up advancement in the bumble bee happens over a 8-to 9-day time frame. Pupal bumble bees at stages 4–6 (P4–P6) of the nine phases of transformative grown-up improvement were gathered from the brood outlines. The phase of advancement was found out by utilizing a strategy like that portrayed by Jay (1962) inviewof outside signs, for example, eye shading and head pigmentation. The investigations portrayed in this work consent to the laws of New Zealand controlling logical research. Essential cell societies were set up from the ALs of pupal honey bees as depicted somewhere else. The heads of 8–10 pupal honey bees were expelled from their bodies, putina dish fixed with sylgard (Dow Corning, Midland, MI), and verified with bug mounting pins. The frontof the head containerand glandulart issue encompassing the mind were expelled. The uncovered cerebrum was flushed with changed Leibovitz L-15 culture medium (BL15, pH7.2,500mOsm) enhanced with 4.0g glucose, 2.5 g fructose, 24.0 gsucrose, and 3.3 gprolineperliter (all obtained from Sigma, St. Louis, MO). ALs were expelled with fine forcep sand set in new BL-15 medium. The rest of the means occurred under sterile conditions.To help separation of the tissue, ALs were presented to a hyperosmotic culture medium (BL-15 enhanced with an extra 20.0g / lsucrose, 580 m Osm) for10 minutes moved into a Ca2+-and sans mg2+ bumble bee Ringer (inmM: 135NaCl, 5KCl, and 114.5 Tris- HCl; pH7.2,460 m Osm) for a similar period.The tissue was flushed quickly in culture medium (BL-15),over a bundance liquid was evacuated, and the ALs (6/250µl) were separated by trituration.The separated cells were moved in 100- µlaliquots to the focal point of anun coated plastic culturedish (Falcon, 3001) and permitted to hold fast to the substrate for 3–5 min. The dishes were loaded up with 2ml of culture medium (BL15) and set in a humidified hatchery at 28°C. Cells were kept up in culture for 4–5 days be for use.

Fixclip accounts in entire cell arrangement were utilized to look at DA regulation of ionic flows in bumble bee AL neurons in vitro. The voltage – gated and Ca2+- subordinate flows in spected in this examination have been depicted in detail somewhere else. These flows incorporate a transient A-type K+ current (IA), a continued, deferredrectifier - like current (IKV), Ca2+- enacted K+flows (IKCa), a Ca2+ current (ICa), and a quickly initiating transient TTX- delicate current conveyed by Na+ (INa). Cells were seenunder an IMT-2 magnifying lens (Olympus) utilizing stage differentiate optics. All investigations were directed at room temperature. Recording cathodes (2–3Mω) were set up from borosilicate glass (100-

µlmicropipettes, 1.71 mm OD, 1.32 mm ID; VWR Scientific, West Chester, CA) utilizing a Flaming – Brown micro pipette puller (P-87, Sutter Instruments) and in layed with an answer containing (inmM)100K - aspartate, 40 KF, 20KCl, 2.5MgCl2, 1EGTA,160 sucrose, and 10HEPES (pH7.2). Allthrough the chronicle timef rame, cells were cease lessly very combined with counter feit creepy crawly saline (AIS) containing (inmM)130NaCl, 6KCl, 4MgCl2, 5CaCl2, 160 sucrose, 25 glucose, and 10HEPES / NaOH (pH7.2, 500mOsm). Intersection possibilities were invalidated be foreseal arrangement. Chronicles were made utilizing an Axopatch1D intensifier related to a CV4 1/100 head stage and Digidata1200 interface (Axon Instruments, Union City, CA). Information were gained utilizing Clamp programming (AxonInstruments, Union City, CA) keep running on a 486 PC. Cells were clipped at a holding capability of −70mV, and depolarizing voltage steps were utilized to actuate voltage – gated directs in the cells. Layer flows were separated at 2kHz utilizinga low-pass four-shaft bessel channel and tested at interimsof 100µs. Inmany accounts, a P/4 convention was utilized for advanced subtraction of direct hole flows and capacitance antiquities. No pay was made for arrangement opposition, and in this manner voltage blunders might be available where flows estimated were vast. Not with standing, arrangement to obstruction blunder sought not influence the focal finishes of this investigation.

RESULT & DISCUSSION :

This investigation is the first to uncover the modulatory activities of DA on ionic flows in bumble bee AL neurons. Our outcomes demonstrate that a critical level of AL neurons are touchy to DA and that DA diminishes the adequacy of Ca2+- actuated K+ flows in these cells.We appear, what's more, that in sort1cells, ICa, IA, and most presumably IKV, stay unblemished within the sight of this a mine. While the character of cells receptive to DA presently can't seem to be obviously settled, our outcomes recomm end that type1cells are probably going to speak to a sub population of near by AL interneurons (LNs). In the Als of the honey bee, there are altogether more LNs than projection (yield) neurons (PNs). Assessments of~4,000 LN sperflap and 800 PNs have been accounted for. That a larger part of thecells invitro displayed type1current profiles recommends along these lines that type 1cells are bound to be LNs than PNs. Examination of the electrophysiological properties of the cells underpins this view. Outward current profiles shown by sort1cells are extraordinarily not the same as those revealed for PNs, which rather look like current profiles saw in this examination in cells of sort 2. Type 1 AL neurons, as opposed to type 2 cells, express a conspicuous A - type current. Our outcomes show plainly that IA stays flawless within the sight of DA, and the energy of the DA - delicate flows recognized in sort 1cells recommend in a round about way that the postponed rectifier-like current, IKV, is likewise unaffected by the nearness of this amine. Conversely, IK Catransient and IKCa supported both appear to be immediate focuses of DA regulation. The active properties of the two DA - touchy flows intently look like those of IKC a transient and IKCa supported, and impacts of DA on sort 1 cells are copied bythe IKC a blocker, CTX. That no recognizable DA balance was seen incells in which IKCa had been hindered with CTX before DA application gives solid help to the end that DA regulates Ca2+-subordinate K+ flows in the seneurons.

Ca2+-subordinate K+ flows have been portrayed in numerous bug species. Transient and continued segments of IKC a have been accounted for, for instance, in cockroachdorsal unpaired middle neurons and, a sin sort 1cells, the two flows are hindered by CTX. The quality encoding the supported IKCadivert in cockroach neurons has been recognized and in cells heterologously communicating this channel, continued IKCa has anenactmentrate like that of the DA-touchy supported current saw in bumble bee AL neurons.

Reports depicting the result of IKC at weak in other invertebrate frame works give intimations as to likely impacts of DA balance ofCa2+-actuated K+flows on the sensitivity of Apis AL neurons. Inmolluscan focal example generator neurons, serotonin-incited lessening of spike after hyperpolarization, ascribed to a decrease in IKCa abundancy, advances dull spiking inthese neurons (Katz and Frost1997). Applying DA to secluded pyloricdilator neurons in the lobster stomato gastric ganglion, then again, expands the sufficiency of complete IKCa adding to an expansion in interspike interim and a decrease in real life potential recurrence in these neurons. These outcomes, together with reports indifferent frameworks, recommend that DA-incited lessening of IKCa will expand the volatility of bumble bee AL neurons.

Increating sensory systems, dimensions of electrical action significantly affect neuronal development and separation and on movement subordinate tuning of neuronalassociations. DA-prompted changes in cell

sensitivity thus lycould have formative pertinence in ALs of the honey bee. The early appearance of DA-immunoreactive procedures in bumble bee ALs, proof that DA-receptor qualities in the honey bee cerebrum are unequivocally formatively managed, and the recognizable proof in this investigation of DA-delicate flows in creating AL neuronsall recommenda formative job for DA, not withstanding its capacities in the grown-up mind. In ALs of the sphinx month, Manduca sexta, modulatory activities of serotonin on creating AL neurons unequivocally anticipate the activities of this a mine in the mind of the grown-up moth.

Ingrown - upbumble bees, olfactory data is encoded by spiking designs in AL projection neurons and examples of action over the glomerular cluster. Bothare probably going to be influenced by DA - instigated changes in the movement of nearby AL inter neuronsor potentially projection neurons. DA has the potential in this way to impact the identification and separation of smells and may be like wise olfactory learning and the arrangement of odotopicre collections. A nearly examination of DA worki n bumble bee ALs demonstrated that DA connected to the projections diminishes the level of creatures that react to an adapted olfactory improvement. One conceivable focus of DA balance is the sub population of LNs that contain the in hibitory synapse GABA.A round 750 of the 4,000 LNs in the bumble bee ALs are GABA immunoreactive, and there is convincing proof that these neurons assume a job in AL capacities, for example, scent segregation. Be that as it may, DA may not target GABA ergic pathways alone. In the vertebrate olfactory globule, for instance, DA directs GABAergic inhibitory preparing, yet in addition control stangible contribution to the olfactory knob through apresynaptic activity on olfactory nerve terminals. DA is probably going toact at different locales likewise in essential olfactory focuses of the bumble bee mind. No less than two DA receptor qualities are communicated by Deuto cerebral neurons that encompass the ALs, and in the sex examination, type1 cells, yet additionally fewsort 2 AL neurons, reacted to thisamine.

While the systems through which DA works inthe ALs still can't seem to be completely settled, the after effects of this investigation give an imperative advance toward this objective.The point off uture examinations will be to decide the practical out comes of DA regulationof Ca2+-enactedK+flows, both inthe creating AL sandin ALneuron sof the grown-up working drone.

CONCLUSION:

One century of investigations has given broad information on the olfactory conduct of bumble bees, on the neuro an atomical association of their olfactory pathway just as on the neura lportrayal of scents inside these circuits. Everyone of these tests agree to demonstrate that the bumble bee olfactory frame workis tuned for playing out various activities that areurgent world wide incrementin glomerular volume was found in matched honey bees inrespect to unpaired honey bees. For everyone of the scholarly smells (here 1-hexanol), three glomeruli demonstrated asignificant volume increment. (C) Counts of microglo meruli numbers in the MB calyx, in view of synapsin / phalloid in twofold recoloring. Olfactory longhaul memory instigated an expansion in microglomeruli numbers in the liparea (olfactory) contrasted with unpaired or credulous honey bees. This longhaul versatility depends on interpretation as infusion of Actinomycin D obstructed the impact. This auxiliary versatility identified with olfactory longhaul memory was coherently discovered just in the calyxlip (olfactory in foarea) and notin the neck line (visual in formation district).
Formeeting thedemandsof social life, food search, and mating. This system thus allows to (1) detect and identify odor stimuli, allowing grade dresponses to increasingly similar odors; (2) measure stimulus concentration allowing both concentration invariant and concentration - specific odor recognition; (3) detect components within a mixtureas wellas extract mixture-unique properties; (4) constantly adapt to the odor our environment; and (5) learn relationships between almost any odor and appetitive or aversive outcomes. Although our understanding of odor representation at the different levels of the bee brain has greatly improved in the last years thanks to state-of-the- art recording techniques, entire brain regions have yet to be explored. The most prominent are them- APT dependent partsof AL and MBs, as wellas the utterlyun studied LH.Thanks to optical imaging, our understanding of the spatial representation of odors has greatly improved, buttemporala spects are still poorly understood. Even in such a simple system, as compared tovertebrates, olfactory coding involves complex interactions between different neuron types, so that only computational approaches feeding on comprehensives ets of experimental data may help understanding the dynamics and processing rules of the olfactory system. Lastly, plasti city appears in multiple regions of the olfactory pathway, but their respective implications for tuning the olfactory system or for storing outcome- related memories is still unknown. It shall be the goal of future research to progress in these questions, so that a

comprehensive model of olfactory detection, processing, and learning in the honey bee can be constructed, the ultimate goal of sensory neuro science.

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