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ROLE OF LOCUST OLFACTORY TO DETECT CANCER

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ABSTRACT: Locusts are well-known for their exceptional sense of smell, are emerging as potential bio-hybrid sensors that could be used in cancer detection. This review analyses the possible ways to develop their sensitive olfactory systems to detect volatile organic compounds (VOCs) released by cancer cells. Around 50,000 receptors remain present on the antennae of locusts, are capable to specify cancerous samples from non-cancerous matters. Recent review discussed about the possibility of coupling locust olfactory with bioelectronic devices or biosensing robotic systems for cancer diagnosis. Locust olfactory receptor neurons (ORNs) are capable to detect fumes of VOCs and send signals through antennal lobes to projection neurons (PNs) where the signals are transformed to identifiable neural patterns. Again olfactory lobes are easy to modify by tissue culture. While the biohybrid concept is still incomplete and faced with issues of scalability and specificity, it has the potential to detect early-stage cancer.

INTRODUCTION: Insects are largest and widely distributed group of animals in the world. In the regulation on different mechanisms of life processes, their diversity acts as a source of incredible variety. Locusts are known for their olfactory abilities. They are increasingly being investigated for their potential anti-cancer properties ¹. This review explores the fascinating world of locusts and their surprising contribution to cancer research. Locusts are insects belonging to the family Acrididae, which is part of the larger group known as grasshoppers. However, locusts differ from typical grasshoppers because of their ability to form large, destructive swarms. These swarms are capable of devastate crops and vegetables over vast areas ².

Here are several types of locusts, mainly classified on their geographic distribution and the regions they affect:



FIG. 1: LOCUST

Desert Locust: The desert locust is a species with short-horned, periodically swarmed grasshopper, belonging to the family Acrididae ³. It is the most notorious species of Africa and the Middle East, and Southwest Asia.

Migratory Locust: Most of the locust species is the migratory locust, genus locusta. Those are

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common in Africa, Asia, Australia, and New Zealand. As it covers very large geographic range as well as numerous diverse ecological zones, so many subspecies of migratory locusts were documented⁴.

Red Locust: The red locust is a large grasshopper from sub-Saharan African distribution. It is named according to the colour of its hind wings. It referred as *Criquetnomade* in French, because it's Nomadic in nature during dry season. When it swarms, it is called locus t⁵.

Australian Plague Locust: The Australian plague locust is a locust species from the family Acrididae. It is indigenous of Australia, where it is a major agricultural pest. Adult Australian plague locusts are 20-45 mm long, with brown to green colour⁶.

The lifespan of locusts varies depending on the species and environmental factors, but generally, it ranges from a few months to a year or more⁷. The typical life stages and their durations as follows:

Egg stage for 2-4 weeks, **Nymph** stage for 4-6 weeks and **Adult** stage for 2-6 months.

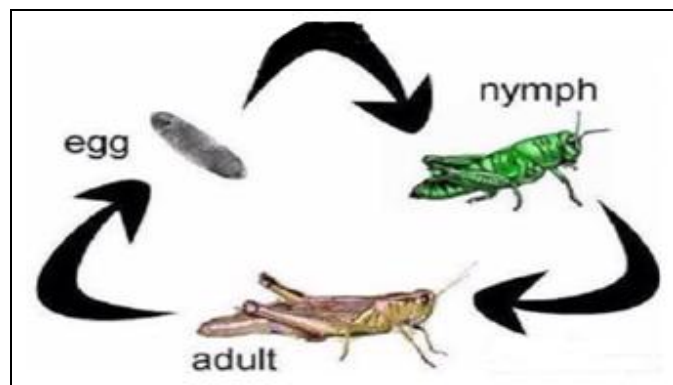


FIG. 2: LIFE STAGES OF LOCUST

The locusts are employed as a model to investigate it's sense of smell, which is processed by the olfactory system, is vital for survival throughout life. It is crucial for behaviors like foraging, predator avoidance, and mate finding. Further, the olfactory system is adapted as an aspects of information processing by networks of neurons. The investigation of sensory processing is relatively simple in the case of insects. During locusts develop from hatchling to adult, they gain experience of the olfactory world, body size increases by an order, and brain size become double. Fresh-hatched locusts without any previous

exposure to food become capable to identify the food odors⁷. Newly hatched locusts have adult-type glomeruli in their Antenna lobes (Als), and their OSNs and Projection neurons (PNs) can response by producing action potentials when odors are introduce into the antennae. So during the contact between the Olfactory receptor neurons (ORNs) with any volatile chemicals, then the antenna of ORNs create a structure into the Als. ORNs and Als are act like receptors and their output gather red into glomeruli of spherical neurophilis. As a result, there produce an excitatory connection between projection neurons (PNs) and local neurons (LNs). Into the gap junction inhibitory and excitatory LNs and PNs synapse interact with each other⁸. The projection of PNs remain spread up to the Mushroom body (MB) and Lateral horn (LH) which has different type of neurons and responsible for detection of various odour. The presence of LH remains observed into the various portions of Locust's brain. Again, neurons present into the MB are known as Kenyon cells (KCs). This KCs synapse remains spread up to a single giant GABAergic neuron (GGN).

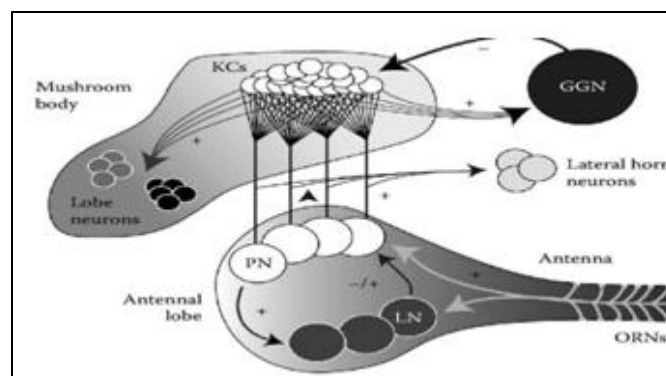


FIG. 3: THE LOCUST OLFACTORY SYSTEM

Locusts have ability to sense odours by using their ultra-sensitive antennae. The sensation streams into the antennal lobe which is a part of the brain with a few hundred neurons. Here nanoparticles were amplifying neural signals. These nanoparticles convert light into heat, which increases the circulation of ions (sodium, potassium), and then release pre-infused hormones like dopamine, serotonin and octopamine into cells. The combined escalation of hormones and ions significantly amplifies the neural response. A locust's antenna contain around 50,000 of olfactory receptors. It is the reason to make it ultrasensitive to smells. An electrode in that antennal lobe, called ultra-

sensitive electronic nose (e-nose), has various potential applications such as detection of explosives and drugs, conduction of bio-diagnosis, monitor the environment, and control industrial processes⁹.

Insect Olfaction:

Locusts as a Model: Insect olfaction is the ability of insects to detect and identify chemical compounds in their environment. Locusts serve as model organisms to study olfactory systems due to their well characterized sensory organs and behaviors¹⁰. Locusts are solitary, but they are more prevalent in some conditions and alter their behavior and way of life and becomes gregarious. Locusts are recognized as swarm-forming, employ olfaction to find food sources as well as to detect pheromones. Researchers were used locust to detect cancer by recording the brain wave patterns of locusts when exposed to gases emitted from cancer specimens¹¹.

Locusts Antennae remains covered with sensory hairs known as sensilla, which respond to odor molecules. Locust antennae possess three types of olfactory sensilla with varying morphology and the number of olfactory sensory neurons (OSNs) present. They were *Sensilla basiconica* (with up to 50 OSNs), *Sensilla trichodea* (with 1-3 OSNs) and *Sensilla coeloconica* (with 1-4 OSNs). There is one sensory neuron per sensillum to convey olfactory data, to the olfactory lobe. The locust olfactory lobe's sensory neurons are olfactory receptor neurons (OSNs), present on antennae. OSNs project axons into the antennal lobe (AL), which synapse onto the dendrites of projection neurons (PNs) and local inhibitory interneurons (LNs)¹².

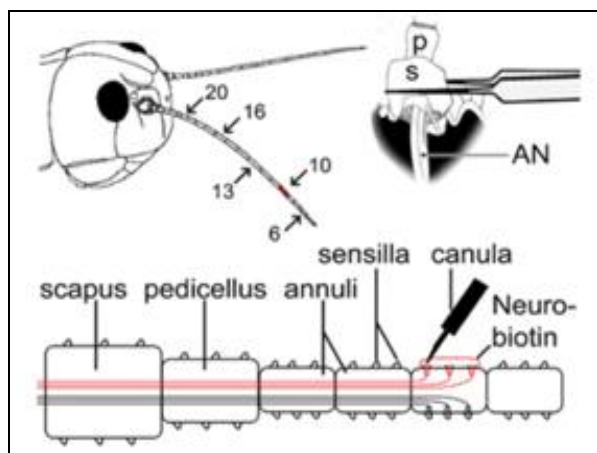


FIG. 4: LOCUST ANTENNAE

Anatomy of Locust Olfactory Lobes: The olfactory lobes of locusts are centered around their antennal lobes. Those are the primary structures responsible for processing olfactory (smell) information. Olfactory lobes are essential to detect and interpret odors. The olfactory system is responsible for the sense of smell, varies greatly across species¹³.

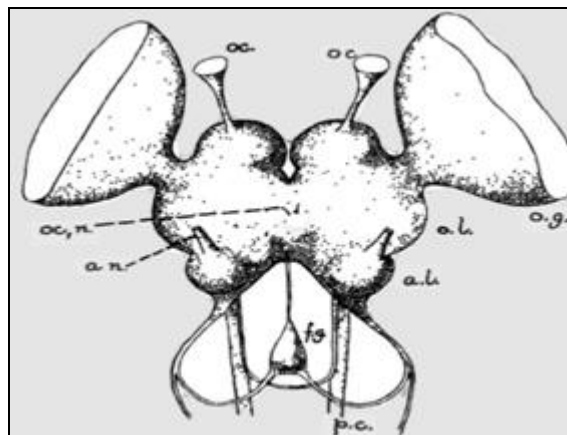


FIG. 5: FRONTAL VIEW OF LOCUST BRAIN. a. l., Olfactory lobe; a. n., Antennary nerve; f. g., Frontal ganglion of Sympathetic system; oc, Lateral ocelli and nerves; oc, n., Nerve to median ocellus; o. l., Optic lobe; o. g., Optic ganglion; p. c., Connective to suboesophageal ganglion.

The olfactory lobes of the locust's brain, are located in the deutocerebrum. Locust's olfactory lobe has three main parts:

Olfactory Glomeruli: These are ball-shaped areas that receive signals from sensory neurons to detect smells. The olfactory glomeruli are made up of:

Olfactory Receptor Neurons: These neurons have branches to catch the smell particles and send signals to the glomeruli.

Glomerular Neurons: These neurons help to process and strengthen the smell signals inside the glomeruli.

Local Interneurons: These neurons connect different glomeruli and help in signal processing by control and enhance the signals.

Olfactory neurons: These neurons process the smell signals and send them to other parts of the brain.

Output Neurons: These neurons carry the processed smell information to different areas of the brain.

Glomeruli and Interneurons: In locust, the major olfactory center of the brain is the antennal lobe, which become larger with a higher number and volume of glomeruli, during development¹⁴.

The glomeruli are innervated by a fixed number of projection neurons that exhibit augmented dendritic arborizations in the course of locust development.

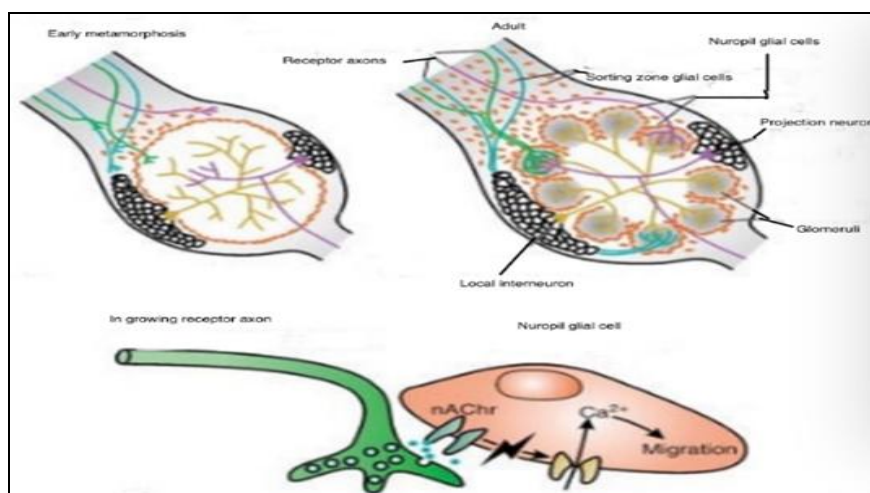


FIG. 6: ANATOMY OF OLFACTORY LOBES OF LOCUST BRAIN

Processing Olfactory Information in Locusts: Neural Pathways: Locust antennae and noses possess specialized epithelia, lined by tens to thousands of ciliated olfactory receptor neurons (ORNs).

Sensory Neurons: The sensory neurons of locusts are specialized cells responsible to detect a range of environmental stimuli, such as touch, sound, smell, and vision. Those neurons translate sensory data into electrical impulses that transfer to the central nervous system, where they are processed and decoded. Sensory neurons transmit signals from the antennae to the olfactory lobe.

Glomeruli: In locusts, the glomeruli of the antennal lobe are central hubs of neural pathways

for olfactory processing. These pathways allow locusts to detect, process, and response to odors, and also influence it's behaviors such as foraging, mating, and escape from predators. Glomeruli integrate signals from multiple sensory neurons, to enhance the olfactory signal.

Interneurons: Interneurons play a critical role in the neural pathways of locusts, bridging sensory input and motor output while integrating information across various sensory modalities. These interneurons allow the locust to coordinate complex behaviors like flight, escape, and foraging. Interneurons relay information to higher brain centers, enabling further processing and behavioral responses.

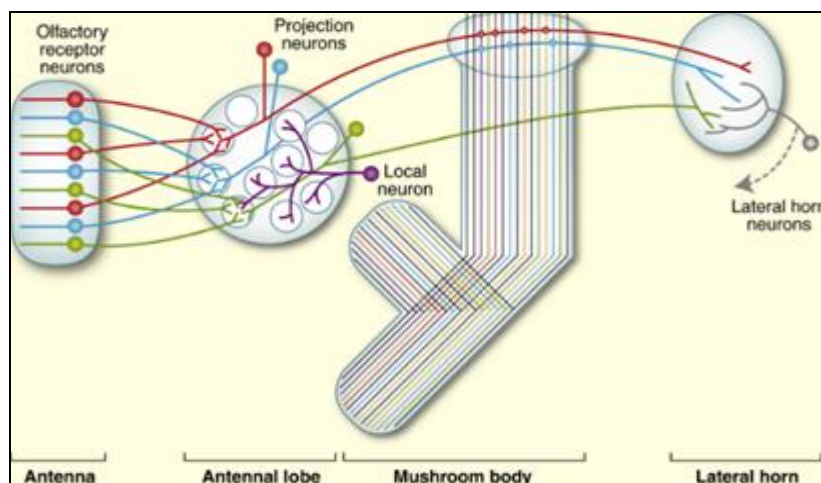


FIG. 7: OLFACTORY INFORMATION PROCESSING

Olfactory processing occurs across several layers of neurons from the transduction of odorants in the periphery, through odor quality processing, learning, and decision making in higher olfactory structures¹⁵.

Tissue Culture-to utilize the Olfactory System of Locust as Cancer Detector: It is proved that the olfactory lobes of locusts are very powerful to detect and process smells. Olfactory system is generally identified by its nature of sensory neuron regeneration. It is expected that there is a chance to increase the number of neurons as well as extension of neurons within few days which can develop a strong cancer detecting agent by utilize the technique of *in-vitro* cell culture¹⁶.

In-vitro cell culture of locust olfactory mucosa involves isolation and neuronal development in an artificial environment that supports their survival and potential growth.

Materials Required: Locust's olfactory mucosa, Dissection tools (scissors, forceps, razor blades, scalpels etc.), Sterile Petri dishes or culture dishes, Sterile culture medium, different type of enzymes and antibiotics as per requirement, Sterile glassware, CO₂ or cold storage to anesthetize the locust, Incubator (set to 25-30°C), Sterile working area (e.g., laminar flow hood).

Culture Medium: General insect tissue culture media such as Grace's or Schneider's medium, Dulbecco's modified eagle medium or Ham's Nutrient Mixture with GlutaMAX contain 1% of antibiotic solution such as penicillin or streptomycin are generally employed in the case of locust brain tissues.

These medias are also needed to supplement with fetal bovine serum (FBS) and vitamins to support neural tissue. An important consideration is to maintain the right osmotic balance and pH 7.2-7.4, for the survival of the tissue¹⁷.

Methodology:

Anesthetization and Dissection of Locust:

- **Anesthetization:** To prevent the movement and reduce the stress during dissection, the locust has to anesthetize by exposure to CO₂ or by chilling it on ice for 5-10 minutes.

- **Dissection:** Under a dissecting microscope, the anesthetized locust has to place on a sterile dissection platform.
- By use fine scissors and forceps, require careful remove of the head from the body.
- Now it is require to open the exoskeleton of the head cautiously by the aid of a fine scissors, to expose the brain, without any damage of brain tissues.
- Once exposed, require to isolate the olfactory lobes which are part of the central nervous system and are locate near the front of the brain. The olfactory lobes are often characterized by their size, shape, and their close proximity to the antennae.
- Now it is require to Careful remove of the mucosa from olfactory lobes of the brain, with minimal damage of the tissue.

Preparation of Locust Brain Tissue Samples:

Under the aseptic conditions to prevent contamination of brain tissue samples of Locust, the olfactory lobes are need to isolate under the guidance of stereomicroscope with the aid of high-precision tools. Then specific enzymes are require to separate olfactory lobe mucosa for culture¹⁸.

Culturing the Olfactory Lobes: After proper dissection, the olfactory mucosa is require to transfer into proper culture media supplemented with about 1% of antibiotic solution and then treat with Hank's Balanced Salt Solution (HBSS) like buffer solution without calcium and magnesium for wash and eliminate excess mucus. Then the mucosa is require to transfer into sterile Petri dishes containing 2 or 10% of fetal bovine serum (FBS) and after that by use a scalpel reduce its size as per requirement.

During incubation require to use enzyme solution like Dispase II solution with serum-free suitable culture media. This type of culture media is essential for digestion of primary cells which remain adhere in culture. Require incubation period is 45 minutes at 37 °C, with 5% CO₂. Then require to triturate and centrifuge of tissue at 400 times g for 5 minutes. That is the process of resuspension of the tissue and cell pellets into the suitable

enzyme solution and then need to incubate at 37 °C, 5% CO₂ for 15 minutes, with trituration in every 5 minutes. Then the resulting solution is require to centrifuge at 400 times g for 5 minutes and after that resuspension of cell pallets are require as before. During incubation need to maintain the appropriate temperature along with humidity as well as sterile environment to prevent any type of microbial growth.

The culture medium is required to keep at a temperature of 25-30°C, which is the natural temperature range for locusts. The medium has to change regularly (e.g., every 2-3 days) to avoid contamination and to provide fresh nutrients.

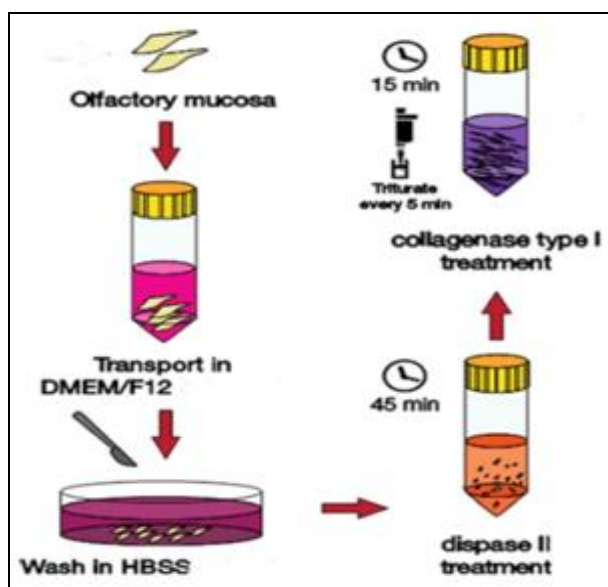


FIG. 8: CULTURE OF THE OLFACTORY MUCOSA

Maintenance of the Culture: Insect neural cultures, particularly from locusts, often require regular supplementation with nutrients. So, it is essential to add fresh medium with serum and growth factors during study about neurogenesis or cellular responses. To establish long-term cultures, we need to subculture the tissue to remove the growing cells or tissue fragments and transfer them into a fresh medium. This can help to maintain healthy cell growth over time¹⁹.

Troubleshooting: It is very much essential to ensure sterile technique during the dissection and culture process, to minimize bacterial or fungal contamination. If contamination occurs, then need to remove the tissue immediately as well as replace the culture medium. If the olfactory lobes show signs of rapid degeneration like tissue breakdown

or lack of growth, then consider the adjustment of culture medium by providing additional supplements like different growth factors or altering environmental factors like temperature or humidity.

Challenges in Studying Locust Olfactory Lobes:

Two essential stimuli for locusts are physical contact in the form of mechanical pressure and olfactory stimulant such as odorants. A locust is able to vomit upon squeeze its head or abdomen. Odorant E-2-hexenal can induce vomiting of locusts²⁰.

As Locust brains are small, require precise dissection and manipulation which is very difficult. Again, Olfactory lobes intricate neural networks that are challenging to study *in-vivo*. In the other hand isolated brain tissues are Kept in living condition and functional in a significant hurdle. Mimicking the natural conditions of the locust brain *in-vitro* is another complex.

It is very essential to ensure that the culture medium and environmental conditions are optimized for the specific requirements of locust tissues. Insect neural cultures, including locusts, who have a shorter lifespan *in-vitro*, compare to mammalian cultures. So quick work or use advance techniques are needed to maintain tissue health over extended periods²¹.

Monitoring Growth and Viability: Periodic inspection of the cultured olfactory mucosa under a microscope is a primary requirement to check the signs of growth, like cell proliferation, or modification of cell morphology. Depending on the culture condition within 2 to 3 days, light or fluorescence microscopy are very essential to observe the cultured tissue, especially their neural outgrowths (axon and dendrite). During the maturation of culture, the development of axonal and dendritic processes, indicate the neuronal health and network formation²².

Again, to assess cell viability, use of vital stains like Trypan blue (dead cells will take up the stain), or use of live/dead assays by Utilizing the Calcein-AM and Ethidiumhomodimer are very useful. Immunohistochemical staining technique is useful to identify specific neural markers like synaptic proteins, ion channels, neurotransmitter receptors

etc. of the cultured tissue. This process is helpful to track the development of different cell types or neuronal connections within the olfactory lobes²³.

In-vitro Growth and Differentiation of Olfactory Lobe Cells: To study on the development and membrane biophysics of cells of the locust's central olfactory pathway, dissociation of the neurons and glial cells from antennal lobes (ALs) and subsequent culture in the *in-vitro* environment of tissue culture are general methods. Supplementation into culture media with particular growth factors such as brain derived neurotrophic factor and nerve growth factor, for neural development is a fundamental requirement²⁴. Utilizing biocompatible scaffolds to mimic the three-dimensional structure of the brain is a very advance idea. This supports more natural cell growth and organization. Again, apply controlled electrical impulses to cultured cells encourages the formation of functional neural networks and synapses²⁵.

Histological Analysis of Cultured Olfactory Lobe Tissue: There are three types of staining methods present in histological analysis of cultured olfactory lobe tissue. They are Nissl Staining, Immunohistochemistry, Golgi Staining.

Nissl Staining: In this staining method the target structures are Neuronal Cell Bodies, and they are visualized as blue purple colour²⁶. Following steps remain involve in this staining technique:

Tissue Preparation: First step of tissue preparation is fixation where the tissues need to be fixed using a fixative such as formalin in order to maintain their structure.

The next step is sectioning where the specimen remains embedded in paraffin and embedded tissue was cut into semi thin sections (around 5–10 μ m thick) by a microtome, rotator, or cryostat after fixation.

Then in staining process the section is glued into a glass slide with an appropriate medium.

RNA, present in Nissl bodies is bound by cresyl violet, a type of Nissl stain. Toluidine blue and thionine are substitutes. Thionine and toluidine blue can also be alternatives. As a general rule of

thumb, tissue is need to place in a solution containing the stain for a certain amount of time and then washto get rid of extra dye. During deeper staining, place the tissue in the solution for longer time.

Differentiation: Wash the muscular system with a dilute alcohol or acid solution to remove extra dye. Right amount of dye concentration is required to achieve the right shade²⁷. That fastens the step where the Nissl bodies become stained while everything else stays relatively clear.

Mounting: After an altered tinge, the specimen becomes dehydrated and then placed it between a coverslip and a mounting medium that maintains specimen integrity and make it easy to store.

Microscopy and Visualization: The filtered coverslips are observed through a bright field microscope, and through bright field microscopy the stained neurons can be visible around the center which are colored darker than the rest.



FIG. 9: NISSEL STAINING

Immunohistochemistry: In this staining method, the target structures are Specific Proteins and they are visualized as Fluorescent colour²⁸. Following steps remain involve in this staining technique:

Tissue Preparation: First step of tissue preparation is fixation where tissue arrays are need to preserve and antigenic components are maintained by cross-linking with formaldehyde or paraformaldehyde. Next is embedding where tissues after fixation are generally encased in paraffin wax or embedding compound optimization for frozen sections. Next step is sectioning where the prepared and encapsulated tissue is slice by a cryostat for frozen sections or microtome in the case of paraffin sections.

Antigen Retrieval (if necessary): Since, formalin-fixed tissues have some obscure epitopes, retrieval of those epitopes are required to expose in the target to increase the chances of antibody binding. In that case HIER and enzymatic treatment of epitopes are required to use.

Blocking: To block non-specific binding sites, in the specified sections, tissues are placed on water bath containing serum to block non-specific binding or antibody cross linking to tissue sections.

Primary Antibody Incubation: Tissue sections are exposed to the primary antibody that is directed towards the target antigen. The time during which the tissues are exposed to the antibodies varies according to tissues and antibodies.

Secondary Antibody Incubation: These tissues are dubbed to secondary antibodies that attach to the primary antibody. Secondary antibody is usually exposed to a Morphund enzyme or chemi-anoin fluorescent²⁹.

Golgi Staining: In this staining method the target structures have Neuronal Morphology, and they are visualized as Black colour³⁰. Following steps remain involved in this staining technique:

Tissue Preparation: This process begins with collection of the preferred tissue. For example brain and spinal cord. If the tissue is not fresh, then it should be preserved in the fixation solution as formalin. The next stage is to cut the specimens into small blocks with dimensions 2 to 3 mm to expedite the process of impregnation.

In the first level of Impregnation tissues are soaked in 2 to 5 percent potassium dichromate solution for about twenty-four to twenty-seven days. During the impregnation process, potassium dichromate is use as the oxidizing agent to prepare the tissue for silver impregnation. Ensure that the solution is stored in a dark area to avoid light. Furthermore, the solution must be changed on daily basis for more effective results.

In the second level of Impregnation tissues are treat with potassium dichromate, and transferred into a solution of silver nitrate with concentration 0.75 to 2 percent for a period of 24 hours to 3 days. In this chemical process, the silver nitrate reacts with the

chromate of the tissue and deposits the crystals of silver chromate. The tissues must be shielded from light to control the consistency of the stain.

Tissues Sectioning: The next step is to offer the support towards the staining and the preparation of tissues for embedding on pellets-to be started by gradual increasing the concentration of ethanol from 70% to 90% and to 100%. This step is a long way to preserve the staining as well as to ensure that the tissues are ready for embedding.

During Paraffin embedding and Sectioning require a microtome cut longitudinal section, between 20-100 m as specimens embedded with paraffin³¹. Finally, tissue can also be embedded in a block of paraffin or directly cut.

The insect olfactory system is construct with thousands of sensory neurones on each antenna, which extend into the main olfactory center, the glomerular antennal lobe.

Functional Assessments of Cultured Olfactory Lobes:

Calcium Imaging: It is a process to visualize neuronal activity in response to olfactory stimuli using fluorescent indicators.

Procedure:

Preparation of Sample: Samples are growing cultures, slicing up tissues or animals, if live imaging is require.

Regarding to chemical dyes, the first step is to load the dye into cells and incubate AM ester dyes with cells. to the GECIs, the most effective way is delivery of gene into the cells with the aid of viral transfection or engineering.

Imaging Setup: A fluorescence microscope (wide-field, confocal, or two-photon) equipped with appropriate filters and detectors is needed. Some of that systems have the potential to create an overlap whereas some systems are live imaging systems which include environmental biospheres for control temperature or gaseous exchange³².

Data Acquisition: A suitable wavelength should be determined by calcium indicators and it's excitant. Time is a factor for the recording of fluorescence signals. There is a requirement to take

the information on intervals of time³³. The other variation like absence or a change in concentrations of calcium ions will reflect the changes in the intensity or shift of fluorescence colours³⁴.

Experimental Assays: Electrophysiological experiments include patch-clamp recordings or field potential measurements are the methods of assay to detect the matured tissues and to study their neuronal activity in response to different stimuli like odorants³⁵.

In stimulus application methodology, cultured tissues become exposed into different olfactory stimuli like odorants or chemicals to study how the olfactory lobes process and response to sensory inputs³⁶. This may involved direct chemical exposure or the use of microfluidic systems to deliver precise stimuli to the tissue.

In case of pharmacological treatment, to study the effects of specific pathway remains involve into olfactory processing and neuronal behavior of culture to introduce various pharmacological agents like ion channel blockers, neurotransmitter receptor antagonists etc³⁷. The processing of the fluorescence data concern about the calcium ions and calcium ion oscillations.

Patch-Clamp Recording measure electrical activity of individual neurons in the cultured tissue.

Procedure:

Preparation: Prepare the tissue slices or cells. Use a pipettes filled with an appropriate intracellular-like solution.

Approach and Seal Formation: Under microscopic guidance, position the pipette against the cell membrane. Apply gentle suction to form a high resistance seal between the pipette and the membrane; giga-ohm seal.

Recording Configuration: Select the appropriate patch-clamp mode, for example cell-attached, whole-cell.

Data Acquisition: Apply voltage or current protocols by the amplifier. Record the resulting ionic currents or membrane potentials³⁸.

Analysis: Analyze data for channel properties, including conductance, gating kinetics, and

responses of ligands or voltage changes. In the other hand Neurotransmitter assays analyze the release of specific neurotransmitters associated with olfactory processing³⁹. In the development of a neurotransmitter analysis for the cultured closed system of geese, there has several steps to isolate, analyze, and quantify neurotransmitters from closed structures, such as antennal wings, which are responsible for processing closed signals.

Materials Required:

- Grasshopper (should be a newborn child or young adult)
- Culture medium (such as Leibovitz's L-15 cell culture medium or other suitable insect cell culture medium)
- Dissection tools (scissors, forceps, dissecting microscope)
- Standard neurotransmitters (such as acetylcholine, glutamate, GABA, dopamine, serotonin)
- Reagents for kidney media extraction:
 - Phosphate buffered saline (PBS)
 - Dissolve 0.1 M perchloric acid (HClO₄) or other suitable extraction buffer.
 - Sodium bisulfite 0.1 % (to prevent oxidation of neurotransmitters)
- Centrifuge and microcentrifuge
- Chromatography or detection systems (such as HPLC with electrochemical detection Mass spectrometry or fluorescence detection)
- Pipette and micro pipette bit

General Regulations:

Preparing the Planting Closure System: Cut:

- Disassemble the grasshopper and remove it's head.
- Use a microscope for careful dissection and open the exposed system (i.e. antenna and antenna cover) under sterile conditions.

- Ensure that dissected Vevet is store in ice-cold PBS or appropriate medium to maintain cell viability.

Culture: Place the closed membrane in the cell culture dish along with the cell culture medium. Store at the appropriate temperature (usually 27 °C) for the specified period. This can vary from several hours to several days depending on temperature. Again, Gene Expression Analysis can examine the expression of olfactory-related genes in cultured cells. Gene expression analysis of closed viticulture systems involves several key steps⁴⁰.

Procedure:

Closed System Sample Collection and Cultivation: Dissection of the olfactory system: The grasshopper's olfactory system mainly consists of antennae and associated sensory neurons in the antennal lobes. Analyze open systems under a 3D microscope and look for minimal contamination or damage to the weft.

In-vitro Culture: After dissection it should keep in a closed system that is a test tube, for growth. This is required to feed with veterinarian prepare such as DMEM (Dulbeccos Modified Eagle Medium) supplemented with essential nutrients and growth factors. To maintain viability and promote physiological activities, gene expression studies are require.

RNA Extraction:

Liquid Homogenization: Homogenizes the open cultured system (antennas and sensory neurons) by using a motorized or stand-mounted homogenizer. This step separates the building blocks å to release the RNA for analysis.

RNA Isolation: Use a commercial RNA extraction kit (such as TRIzol reagent or RNeasy kit) to extract RNA. This removes cell rust and other contaminants. As a result pure RNA becomes isolated.

RNA Quality Check: Quantify RNA using spectrophotometry (NanoDrop) and check its integrity by pass the RNA on an agarose gel or use an RNA analyzer (e.g. Agilents Bioanalyzer)

cDNA Synthesis:

Reverse Transcription: To evaluate transcription at the mRNA level, required to synthesize

complementary DNA (cDNA) from the isolated RNA. For this a reverse transcription kit is required. Here reverse-transcription enzymes remains involved to synthesize cDNA.

Data Analysis: Detection of neuronal behavior is possible to analyze the response of the cultured olfactory neurons against stimuli and assess changes in cell morphology, synaptic formation, and functional activity. Again, use the techniques like qPCR, Western blotting, or IHC, it is possible to measure the expression of specific genes or proteins in response to different stimuli or treatments⁴¹.

Cancer Cell: Cancer cells are intriguing and intricate. These renegade cells, which have abandoned normal patterns of growth, are a primary health issue. Study about their biology is the central challenge to achieve successful treatments. Cancer deviate normal cellular metabolism and these changes are the cause of the production of numerous volatile compounds⁴². Several *in-vitro* experiments, use various permutations of mass spectrometry, have demonstrated that cancer modifies the VOC profiles. VOCs are released by the cell cultures⁴³.

Anatomy of Cancer Cell: - Cellular Components:

- **Cell Membrane:** Altered membrane structures and function resulting increased permeability.
- **Cytoskeleton:** Disrupted organization which affect the cell shape and movement.
- **Nucleus:** Enlarged, irregular shape with increased DNA content.
- **Mitochondria:** Altered function along with reduced energy production.
- **Endoplasmic Reticulum (ER):** Expanded and involved in protein synthesis.

Morphological changes of cancer cells include irregular shape and size, increased nuclear-to-cytoplasmic ratio, loss of polarity, abnormal cell division (mitosis) and formation of pseudopodia (cancer cell migration). Again, some subcellular features of cancer cells are chromosomal instability (aneuploidy), epigenetic alterations (DNA

methylation, histone modification), overexpression of oncogenes, mutations in tumor suppressor genes and altered metabolic pathways (Warburg effect)⁴⁴.

In case of cancer cell reduce its E-cadherin due to loss of cell adhesion. Here cell migration and invasion becomes increased. Also, in case of cancer cells there are several new blood vessels become formed, called the process angiogenesis⁴⁵. Also, here immune evasion mechanisms are occurred.

Structure of Cancer Cells:

Nuclear Alterations: Cancer cells often display abnormal nuclear shapes and sizes, reflecting uncontrolled DNA replication and potential mutations⁴⁶.

Cytoplasmic Changes: The cytoplasm present in the space between the nucleus and cell membrane, shows changes in organelles and proteins, affecting cell function and communication⁴⁷.

Abnormal Cell Membrane: The cell membrane, regulate enters and exits of components from the cell, can exhibit alterations in receptors and proteins, influencing on the growth and interaction of cells with the environment⁴⁸.

Types of Cancer Cells⁴⁹:

Carcinoma (Epithelial Origin): Carcinoma is a kind of cancer found in epithelial tissue, that covers the body's skin, organs, and internal body passages⁵⁰.

Sarcoma (mesenchymal origin): Sarcoma represents an uncommon but highly malignant form of cancer that arises from mesenchymal tissues, including bone, cartilage, muscle, and fat⁵¹.

Leukemia (Blood Cell Origin): Leukemia begins when the DNA of a single bone marrow cell mutates. DNA is the "instructions code" for a cell during growth, maturation and death. Due to the mutation, or coding mistake, leukemia cells continue to divide⁵².

Lymphoma (Lymphoid Origin): Lymphoma is a type of cancer that starts in the lymphatic system, which is a series of tissues, organs, and vessels that assists the body infighting infection. It begins when normal cells within the lymphatic system become

altered and grow uncontrollably. Lymphoma is sometimes referred to as malignant lymphoma or lymphosarcoma⁵³.

Cancer Cell Characteristics:

- Uncontrolled growth and proliferation
- Resistance to apoptosis (programmed cell death)
- Increased metastatic potential
- Genetic instability
- Altered cellular metabolism

Development of Cancer Cell: Cancer is a disease caused by cells in the body multiplying and growing without control. That is the result of failure of the regular cell division and Increase in the number of abnormal cells as well as their proliferation⁵⁴.

The development of cancer is a multistep process that involves:

Tumor Initiation: A genetic alteration caused by a single cell abnormally proliferate.

Clonal Selection: Mutations occur within the tumor population, which is a selective advantage for some cells. For example, a cell with mutation, that allows it to grow faster and becomes dominant in the tumor population⁵⁵.

Tumor progression: The tumor continues to grow and becomes more malignant as additional mutations occur⁵⁶.

Cancer Cells Behave Differently from Normal Cells:

- They don't know when to stop replicating and die.
- They don't always stick together.
- They are able to escape and travel through the blood or lymphatic circuit to other regions of the body⁵⁷.

Released Chemicals from Cancer Cells: Cancer cells release a variety of chemicals, including:

Acrolein: A molecule that cancer cells produce naturally. That acrolein can activate a prodrug, which is a modified, benign form of a medicine⁵⁸.

Cytokines: Substances that have the ability to make a human being unwell, including fevers, chills, sweats, tiredness, anorexia, nausea, and vomiting. Such chemicals are comparable to those emitted when an individual has the flu.

Proteases, Glycosidases, and Collagenases: These chemicals can degrade components of the matrix, allowing tumor cells to invade tissue barriers and blood vessels and lymph channel walls⁵⁹.

Lactate: A partially broken-down type of glucose that is utilizable for the cancer cells to drive biochemical processes and synthesize other substances require for cell growth. Cancer can develop from DNA damage, mutations, or loss of function in regulatory systems. Environmental factors can also play a role, such as exposure to UV light from the sun or radical oxygens⁶⁰.

Visualizing the Growth and Division of Cancer Cells:

Uncontrolled Proliferation: Cancer cells overcome normal growth checkpoints and divide unchecked to produce tumors⁶¹.

Evading Apoptosis: Cancer cells evade programmed cell death, enabling them to survive and accumulate, which leads to tumor growth⁶².

Angiogenesis: Cancer cells trigger the development of new blood vessels to supply oxygen and nutrients to promote additional growth and dissemination⁶³.

Function of Robot to Develop Biosensor on the Basis of Olfactory Lobes: A comparatively uncomplicated insect olfactory system consists thousands of copies of each type of olfactory receptor neuron, which provides a large and diverse set of transducers into the biological system⁶⁴. Even a small insect may have an advanced chemical sensing device in contrast to cutting-edge engineered e-noses⁶⁵.

Robot's Role: Robots can act as accuracy and precision enhancer during detection of odorous

chemicals through Locust engineered e-noses. By using the robotic activities it will be easy to Improve the efficiency and speed of signaling into the olfactory of locust as well as reproducibility. The main advantage of robot is Scalability and automation by Reducing human error⁶⁶.

Biosensor Development: Technical Requirements⁶⁷:

- Microscopy and imaging systems
- Micromanipulation tools
- Electrophysiology equipment
- Sensor technology (e.g., MEA, FET)
- Machine learning software

Steps Involved: Firstly required Locust Olfactory Lobe-Based sensor design with Microelectrode Array (MEA) fabrication, which is essential for Neural Interface Development. Next signal processing and amplification which will help the Integration with Machine Learning Algorithms⁶⁸.

Challenges:

- Handling fragile biological tissues
- Maintaining stable culturing conditions
- Integrating olfactory lobes with sensor technology
- Developing robust signal processing algorithms
- Ensuring biosensor reliability and longevity

Potential Applications:

- Explosive detection
- Environmental monitoring (e.g., air quality)
- Medical diagnosis (e.g., disease detection)
- Food safety monitoring
- Quality control in industrial processes⁶⁹.

Collaborative Research Opportunities:

- Neuroscience and neuroengineering

- Biomedical engineering
- Robotics and automation
- Computer science and machine learning
- Biology and biochemistry

Electronic nose (e-nose) Systems utilise certain biological concepts of gas detection, which include combinatorial coding with cross-selective chemical sensors and template-matching for one-shot classification⁷⁰.

CONCLUSION: To detecting the anti-cancer activity based on the olfactory capabilities of locusts highlights a promising avenue in cancer detection and diagnostics. Research has demonstrated that locusts possess an acute sense of smell, capable of detect volatile organic compounds (VOCs) emitted by cancer cells. These VOCs are distinct from healthy cells, enabling locusts to differentiate between cancerous and non-cancerous samples. Cell culture or robotic biosensor of Locust olfactory lobe may improve that type of smell differentiation capabilities of locust.

The use of locust olfaction in cancer research could pave the way for non-invasive, cost-effective, and rapid diagnostic tools. Their sensory mechanisms might be harnessed through biohybrid systems or electronic sensors mimicking their olfactory processes. However, the approach requires further investigation to address challenges like specificity, reproducibility, and scalability for clinical applications. In conclusion, locust olfactory studies represent an innovative step in cancer detection, potentially revolutionizing early diagnosis methods and improving patient outcomes.

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