M.Sc. Interdisciplinary Biosciences
Laboratory Course II

Course Code: JAP 305
Credits: 0-1-3

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NEURO BEHAVIOUR ANALYSIS: Drosophila and mice

A neuroscientist strives to understand nervous system function and neurological disease using molecular, cellular, synaptic, network, computational, and behavioural tools.

Behavioural neuroscience studies provide the organism level information. It involves studying the basis of learning, memory, circadian rhythm, various response to stimuli etc.

Model eukaryotic organisms have long been established to study behaviour, given the similar underlying molecular machinery governing neural signalling and a shorter life-span than human. Peripheral, relatively non-invasive methods with high spatial and temporal resolution such as eye-tracking, brain activity monitoring, pupil dilation, heart rate variance, and skin conductance are gaining popularity in monitoring reactions to alteration in its environment in primate subjects. However, for their ease of availability, upkeep and ethics, two animal models, viz., drosophila and mice will be used for neuro-behaviour analysis. *Drosophila melanogaster*, the fruit fly, has been extensively used as a versatile experimental model for more than a century. Given its cosmopolitan distribution, inexpensive rearing, short generation time, large progeny size, only four pairs of chromosomes in somatic cells, and availability of genome sequence, linkage maps, and mutant genotypes, the fly model is used as a robust model in diverse areas of biomedical research. The mice model system resembles humans very closely, especially in the context of neuro- behaviour. In this course, the following experiments will be attempted:

- An exercise to estimate the tolerance of flies to heat shock for 1 hour at different temperatures. Parameters like fly viability, male fertility, and physiology, viz., susceptibility to other stressors like starvation, upon exposure to rising in temperature above ambient, would be monitored.

- Vortex assay to measure the epileptic behaviour in *Drosophila*. Excess electrical activity in the brain may cause seizures, one-third of which do not respond to available antiepileptics. Upon mechanical shock, the *Drosophila* seizure model would be analysed for threshold and duration of seizure phenotype like leg tremors, jerking, abdominal muscular spasms, wing flapping, and proboscis extension.

- A negative geotaxis (climbing) behaviour assay to quantify motor function changes in *Drosophila* due to disease or neurodegeneration.

- Novel object recognition test. Mice placed in a test box and familiarized with similar objects for different time points would be tested for short-term and long-term recognition memory by replacing one of the familiar objects with novel (different) object during the testing period.

- Open field test to check anxiety and locomotion activity upon sleep deprivation.

PERIPHERAL BLOOD MONONUCLEAR CELL (PBMC) ISOLATION

PBMCs comprise lymphocytes and monocytes, cells important to study immune responses, during vaccine development, and study of disease pathogenesis, its diagnosis, and therapy. They would be isolated from whole blood components such as plasma, platelets, erythrocytes, and granulocytes by density gradient centrifugation using lymphoprep medium, counted and frozen till use.

ANIMAL CELL CULTURE

Hands-on training in aseptic in-vitro culture of animal cells in serum-supplemented defined media. The following assays will be done using the cultured human cells:

Cell Proliferation/ Cytotoxicity Assay: A yellow tetrazolium dye (MTT) is reduced to purple formazan in metabolically active, live-cells by mitochondrial reductase activity. Upon incubation with the drug, cells seeded (in a 96-well plate) may either show cell death, enhanced growth or quiescence. The formazan crystals upon dissolution in DMSO would be read spectrophotometrically to give the live cell count.

Autophagy assays: Autophagy is the lysosomal degradation of unwanted cellular cargo during stress such as infection, hypoxia or starvation, excess of which can lead to physiological cell death. It involves stepwise initiation, nucleation, elongation and fusion of acidic vesicular organelles to phagophore. The conversion of LC3 protein to LC3II by autophagy-related proteins for its incorporation into phagophore, results in autophagosome which fuses with a lysosome to give autophagolysosome. Acridine orange staining and LC3II expression by Western would be used to analyse autophagy.

Apoptosis assays: Apoptosis is physiological cell death or programmed cell death caused by excess cellular damage or toxin accumulation, mediated by activation of serine proteases called caspases that in turn cleave endonucleases or proteases. These lead to the degradation of nucleoskeletal Lamins and PARP-1, resulting in cell shrinkage, chromatin condensation, nuclear blebbing, formation of micronuclei, and membrane-bound apoptotic bodies. Apoptosis may be intrinsic or extrinsic depending on whether it's initiated by mitochondrial or cell membrane signals, respectively. Former commences upon reduction in mitochondrial antiapoptotic Bcl-2 protein level due to inward movement of pro-apoptotic Bax and Bid from the cytosol, resulting in loss of mitochondrial membrane potential and increase in permeability, causing release of cytochrome C to cytoplasm and formation of apoptotic effector APAF-1-CytC-Caspase9 complex. Later activates when a ligand like TNFα, FasL, TRAIL or TWEAK binds its appropriate receptor, viz. TNFR1/2, Fas or DR-3/4/5, respectively, causing receptor

oligomerization and initiation of death receptor pathway by adaptor protein recruitment and caspase activation.

Upon loss of mitochondrial membrane potential, Rh123 fluorescence reduces. Apoptosis would be detected by measurement of mitochondrial membrane potential by rhodamine-123 and propidium iodide staining and FACS-acquisition, as well as by Hoeschst staining.

Terminal deoxynucleotidyl transferase dUTP nick end labelling (TUNEL) Assay using fluorophore/ hapten modified dUTPs would be utilized to detect apoptotic programmed cell death or excess DNA fragmentation in individual cells.

ENZYME LINKED IMMUNOSORBENT ASSAY (ELISA):

ELISA is an analytical biochemical assay used for the quantitative detection of an antigen using antibody directed against it. First, antigen may be attached to a surface, probed by an antibody, followed by substrate addition resulting in signal which is recorded by color change.

HIV would be detected in patient blood sample using "Sandwich ELISA". Briefly, microtiter plate coated with HIV-1 envelope proteins gp41, and C-terminus of gp120, HIV-2 protein gp36, and anti-HIV-1 p24 antibodies from J. Mitra kit would be used. After incubation of specimen and controls for binding of relevant antibodies to cognate antigens, and vice-versa adsorbed on well surface, the plate would be washed to remove the unbound material. Then, horseradish peroxidase (HRP) conjugated gp41, and C-terminus of gp120 of HIV-1 and gp36 of HIV-2 proteins, and anti-HIV-1 p24 antibodies would be added. Finally, substrate solution containing chromogen and hydrogen peroxide would be added and incubated. The reaction would be stopped by a stop solution and read at 450nm for blue color development in proportion to amount of HIV-1 and/ or HIV-2 antibodies present in the specimen.

FLUORESCENT ACTIVATED CELL SORTING ANALYSIS (FACS):

FACS is an advanced flow cytometry-based technique in which cells labelled, either using a dye, reporter or fluorophore conjugated antibody, could be counted, and sorted into subpopulations. Herein, cells may be identified and separated by virtue of specific expression of cell surface and/ or intracellular molecules, size and morphology.

Propidium iodide would be used to analyse yeast cell cycle using FACS analysis on BD FACS-Aria instrument in a workshop mode.

LIFE SPAN ANALYSIS OF *Caenorhabditis elegans*

Life cycle analysis of *C. elegans* provides insight into life process effected by the environment. Post-fertilization and embryo development within the egg to 558 nuclei stage will be studied. In *C elegans* L1 larva hatches after approximately 16h at 20^{o}C. Three more larval stages (L2-L4) are 12 h long each and are followed by the birth of progeny. The reproductive period starts 12 h post L4 molt and lasts 2-3 days, post which they can live up to three more weeks before dying of senescence. These stages will be demonstrated

Effect of diet alteration on the growth, development and reproduction of *C. elegans,* i.e., using either *Escherichia coli* OP50 or *Pseudomonas aeruginosa* will be monitored.

MODULATION OF EPIGENETIC MARKS

Genes and their interaction with the environment determine phenotype and behaviour. Epigenetic information is encoded by the chemical and structural modifications of chromatin without changing underlying DNA sequence. DNA methylation, covalent modifications of histones, histone variants, nuclear architecture, and the non-coding RNAs constitute the primary epigenetic landscape.

Histone modifications will be studied using recombinant enzymes and cell lines.

References and further reading:

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