

Lecture 1.1: Neurodevelopment, Cerebrospinal Fluid Dynamics, Cilia Biology, and Hydrocephalus

1. Neurodevelopment and Ventricular Morphogenesis

- **Overview of Neurodevelopment:**
 - Neurodevelopment encompasses the formation, patterning, and cellular differentiation of the central nervous system (CNS).
 - **Neurogenesis:** The process whereby neuroepithelial stem cells proliferate and differentiate into neural progenitor cells, which subsequently commit to mature lineages including neurons, astrocytes, and other specialized glial cells.
- **Embryonic Formation of the Ventricular System:**
 - Early CNS development begins with the invagination and folding of the neuroepithelium to form the **neural tube**, an enclosed fluid-filled tubular structure.
 - Differential expansion and patterning of the neural tube give rise to interconnected brain cavities that mature into the cerebral ventricular system:
 - **Lateral Ventricles (paired):** Located in the telencephalon.
 - **Third Ventricle:** Located along the midline within the diencephalon.
 - **Cerebral Aqueduct (Aqueduct of Sylvius):** A narrow conduit traversing the midbrain; highly vulnerable to occlusion by congenital stenosis, cellular debris, or local compression.
 - **Fourth Ventricle:** Located between the cerebellum, pons, and medulla, continuous caudally with the central canal of the spinal cord.
- **Developmental Timing and Structural Proportions:**
 - During early embryonic stages, the ventricles occupy a relatively large proportion of total brain volume compared to the surrounding neural parenchyma.
 - Active neuroepithelial proliferation (marked by cell cycle proteins such as Proliferating Cell Nuclear Antigen [PCNA]) drives cellular expansion from the ventricular lining outward into the cortical mantle.

2. Cerebrospinal Fluid (CSF) Physiology & Dynamics

- **Composition and Physiological Functions:**
 - **Mechanical Protection:** Provides physical buoyancy, cushioning the brain and spinal cord from mechanical trauma.
 - **Homeostatic & Metabolic Maintenance:** Facilitates the clearance of metabolic waste products, distributes nutrients, and regulates intracranial pressure.
 - **Signaling Milieu:** Similar to blood plasma but with lower total protein content (lacking high-abundance heavy proteins like albumin or transferrin); rich in sodium ions, signaling peptides, and essential neurogenic growth factors (e.g., bone morphogenetic proteins [BMPs], fibroblast growth factors [FGFs]).

Lecture 2.1: Neurodevelopment, Neuronal Migration, Axonal Guidance, and Cortical Malformations

1. Fundamentals of Neurodevelopment & the Excitation/Inhibition (E/I) Balance

A. Clinical Significance of Developmental Neurobiology

- **Neurodevelopmental Disorders:** Conditions such as Autism Spectrum Disorder (ASD), intellectual disabilities, and congenital/developmental epilepsies originate from early perturbations in neural stem cell proliferation, fate specification, migration, or circuit formation.
- **The E/I Balance Hypothesis (Rubenstein Model):**
 - Normal cerebral cortical function requires a homeostatic balance between excitatory glutamatergic neurotransmission and inhibitory GABAergic neurotransmission.
 - Approximately 20% to 25% of individuals with ASD exhibit co-occurring clinical seizure disorders, reflecting underlying hyperexcitability or defective inhibitory circuit maturation.
 - **Metabolic & Transcriptional Interdependence:** Glutamate is biochemically decarboxylated into GABA via the rate-limiting enzymes glutamic acid decarboxylase 65 and 67 (GAD65/GAD67). These enzymatic drivers are directly transcriptionally regulated by developmental transcription factors (e.g., the DLX homeobox family). Disrupting these regulatory networks alters the E/I ratio, predisposing the brain to epileptogenesis and behavioral abnormalities.

B. Sequential Phases of Neural Development

1. **Neural Induction:** Conversion of embryonic dorsal ectoderm into the specialized pseudostratified neuroepithelial sheet (neural plate).
2. **Neurulation & Primary Axis Patterning:** Invagination and closure of the neural plate into the neural tube, followed by rostrocaudal (anterior–posterior) and dorsoventral regionalization into forebrain (prosencephalon), midbrain (mesencephalon), hindbrain (rhombencephalon), and spinal cord.
3. **Neurogenesis & Gliogenesis:** Proliferation and diversification of multipotent neural stem and progenitor cells (NSPCs).
4. **Programmed Cell Death (Apoptosis):** Developmental culling of excess or improperly integrated neurons mediated by neurotrophic factor competition.
5. **Neuronal Migration:** Directed movement of immature neuroblasts from proliferative zones to designated anatomical layers or nuclei.
6. **Axon Guidance & Target Recognition:** Extension of navigated growth cones across long and short distances to identify synaptic partners.
7. **Synaptogenesis & Circuit Refinement:** Assembly of functional synapses, dendritic arborization, and activity-dependent synaptic pruning.

Lecture 2.2: Schizophrenia Neurodevelopment, Interneuron Dysfunction, and Circuit Modeling

1. Neurodevelopmental Trajectory and the Excitation/Inhibition (E/I) Balance

A. Foundations of Cortical Neurodevelopment

- **Neurogenesis and Fate Specification:** Cortical neurogenesis begins during early embryonic gestation. Progenitor cells in the proliferative ventricular zones proliferate and differentiate into two primary functional classes of neurons:
 - **Pyramidal Neurons:** Large, excitatory projection neurons (~80% of cortical neurons) with triangular cell bodies, extensive apical and basal dendritic trees covered in dendritic spines (the primary sites of excitatory synaptic inputs), and long projecting axons that travel through the subcortical white matter to distal brain regions or the contralateral hemisphere.
 - **Interneurons:** Morphologically diverse, inhibitory local-circuit neurons (~20% of cortical neurons) that release gamma-aminobutyric acid (GABA) to modulate and restrain the activity of local pyramidal networks.
- **Circuit Refinement and Pruning:** Following initial migration and synapse formation, excess or non-functional synaptic connections are systematically pruned, while active, functionally integrated circuits are stabilized.

B. The Excitation/Inhibition (E/I) Imbalance Hypothesis

- **Homeostatic Control:** Normal cortical cognitive processing requires tight coordination between glutamatergic excitation and GABAergic inhibition.
- **Pathological Shift in Schizophrenia:** Postmortem neuropathology and genetic models demonstrate an intrinsic deficit in interneuron numbers and function. When inhibitory interneurons fail to place sufficient "brakes" on pyramidal neurons, local microcircuits shift toward excessive excitation and desynchronized network activity.
- **The "Two-Hit" Etiological Model:**
 - *First Hit (Prenatal):* Genetic susceptibility (polygenic variants or rare copy number variations) combined with early in utero environmental insults (maternal infection, severe pyrexia, nutritional deficiency, maternal immune activation, or extreme gestational stress).
 - *Second Hit (Late Adolescence / Early Adulthood):* While projection neurons assemble prenatally and in early infancy, cortical GABAergic interneuron connectivity and synaptic properties do not fully mature until late adolescence. Secondary maturational insults or neurodevelopmental failures during this adolescent window trigger clinical symptom onset.

4. The Protein-Only Hypothesis & Molecular Characteristics

A. Development of the Hypothesis

- Physical and chemical inactivation experiments on infected brain homogenates showed that infectivity resists nucleases, ultraviolet radiation, and nucleic acid-damaging procedures. Conversely, infectivity is reduced by proteases, protein denaturants, and strong alkaline hydrolysis.
- Stanley Prusiner (1982) coined the term **prion** (proteinaceous infectious particle) and won the Nobel Prize in 1997.
- Bruce Chesebro demonstrated that *PRNP* mRNA is present in identical sequences in both healthy and infected brains, confirming that prions are conformational isoforms of a host-encoded protein rather than foreign agents.

B. Biochemical Comparison: PrP^C vs. PrP^{Sc}

Property	Normal Cellular Prion Protein (PrP ^C)	Pathogenic Scrapie Isoform (PrP ^{Sc})
Secondary Structure	Predominantly α -helical (~42% α , ~3% β)	Enriched in β -sheets (~30% α , ~43% β)
Protease Susceptibility	Completely digested by Proteinase K (PK)	Partially resistant; PK cleaves N-terminus, leaving a resistant core
Solubility	Soluble in non-denaturing detergents	Insoluble; self-assembles into fibrillar aggregates
Infectivity	Non-infectious	Infectious; templates conformational conversion
Membrane Anchorage	Glycosylphosphatidylinositol (GPI) anchor	GPI-anchored aggregates or anchorless fibrils

C. The *PRNP* Locus and Key Polymorphisms

- Primary sequence: 253 amino acids (processed to 209), containing two N-linked glycosylation sites (un-, mono-, and di-glycosylated forms).
- Codon 129 Polymorphism:** Encodes either Methionine (M) or Valine (V). Strongly modulates genetic susceptibility and incubation times.
 - Example:* The D178N mutation causes **FFI** when paired with a Methionine at codon 129 (178N/129M), but causes **fCJD** when paired with a Valine (178N/129V).

2. Cardiac Excitation-Contraction Coupling and Bioenergetics

A. Calcium Dynamics and Electromechanical Coupling

- **Action Potential and Influx:** Membrane depolarization propagates across the sarcolemma and deep into transverse tubules (T-tubules), activating voltage-gated L-type calcium channels ($I_{Ca,L}$).
- **Calcium-Induced Calcium Release (CICR):** The initial calcium influx triggers the opening of ryanodine receptors on the sarcoplasmic reticulum (SR), releasing high concentrations of stored Ca^{2+} into the cytosol.
- **Cross-Bridge Cycling (Systole):** Cytosolic Ca^{2+} binds to troponin C on the thin actin filaments, exposing myosin-binding sites. Actin-myosin cross-bridges form, generating mechanical force and cardiomyocyte shortening.
- **Calcium Removal and Relaxation (Diastole):**
 - **SERCA (Sarcoplasmic/Endoplasmic Reticulum Ca^{2+} -ATPase):** Pumps Ca^{2+} back into the SR lumen against a concentration gradient using ATP. **SERCA activity accounts for ~60% of total basal cardiomyocyte energy expenditure.**
 - **NCX (Sodium-Calcium Exchanger):** Transports one Ca^{2+} ion out of the cell in exchange for three Na^+ ions moving inward down their electrochemical gradient.
 - **Na^+/K^+ -ATPase & Sarcolemmal Ca^{2+} -ATPase:** Maintain membrane potential and ionic gradients.
- **Energy Consumption:** Electromechanical coupling accounts for roughly **80% of total myocardial energy expenditure.**

B. Substrate Utilization and Compartmentalized ATP

- **Fuel Distribution:** The healthy myocardium utilizes a flexible mix of metabolic substrates: ~65% free fatty acids (shifting up to 90% under high metabolic demand) and ~30% glucose/lactate.
- **Cytoplasmic Glycolytic Compartmentalization:** Membrane-bound ion channels and excitation-contraction coupling machinery preferentially utilize **ATP generated locally via cytoplasmic glycolysis**, rather than the larger pool of ATP produced within mitochondria.

C. Diastolic Dysfunction in Diabetes

- Approximately **75% of diabetic patients exhibit diastolic dysfunction** (impaired ventricular relaxation and filling) in the absence of hypertension or coronary obstruction.
- Impaired myocardial relaxation reduces end-diastolic filling volume, diminishing stroke volume and driving heart failure with preserved ejection fraction (HFpEF).

6. Clinical Spectrum of HTLV-1-Associated Diseases

- **Proviral Load as a Biomarker:**
 - **Proviral Load (PVL):** The percentage of peripheral blood mononuclear cells (PBMCs) harboring integrated proviral DNA.
 - While many carriers remain asymptomatic, individuals with a PVL exceeding 1% face heightened risks of systemic inflammatory diseases, malignant transformation, and early all-cause mortality.
- **Adult T-Cell Leukemia/Lymphoma (ATL):**
 - A neoplastic proliferation of mature CD4⁺CD25⁺ T cells that occurs in roughly 5% of infected individuals after a latency period lasting 30 to 50 years.
 - Diagnosis requires confirmed presence of the integrated HTLV-1 provirus.
 - Peripheral blood films display characteristic neoplastic lymphocytes with polylobulated nuclei resembling petals ("flower cells").
 - ATL responds poorly to conventional systemic chemotherapy; the median survival for acute and lymphomatous variants is less than one year.
- **HTLV-1-Associated Myelopathy / Tropical Spastic Paraparesis (HAM/TSP):**
 - A chronic neuroinflammatory disorder occurring in approximately 4% of infected individuals.
 - Proviral-bearing T cells infiltrate the thoracic spinal cord, releasing pro-inflammatory cytokines that cause bystander demyelination, axonal injury, and meningeal thickening.
 - Manifests as progressive spastic weakness of the lower extremities, gait instability, hyperreflexia, neurogenic bladder dysfunction, constipation, and erectile dysfunction.
- **Systemic Inflammatory and Pulmonary Manifestations in Central Australia:**
 - While classical ATL and HAM/TSP occur in Central Australia, patients in this region frequently present with chronic pulmonary complications.
 - **HTLV-1-Associated Pulmonary Disease (Bronchiectasis):** High proviral loads drive the accumulation of activated, non-functional T cells in the lungs. Chronic cytokine secretion and recurrent secondary bacterial infections cause progressive bronchial dilation, extensive pulmonary fibrosis, and respiratory failure in young adults.
 - **Infective Dermatitis:** Severe, recurrent exudative eczema affecting children, often serving as an early indicator of subsequent ATL or HAM/TSP development.
 - **Additional Associations:** Chronic uveitis, inflammatory myositis, non-destructive polyarthritis, and hyper-infection syndrome with the intestinal nematode *Strongyloides stercoralis*.

5. Immune Dynamics, Perivascular Trapping, and Glioblastoma Microenvironments

- **Cytotoxic T Lymphocyte (CTL) Destruction Mechanisms:**
 - Host anti-tumor immunity depends largely on CD8+ cytotoxic T lymphocytes recognizing specific tumor-derived peptide antigens presented on Major Histocompatibility Complex (MHC) Class I molecules.
 - CTLs kill target cells through two primary pathways:
 1. *Granule Exocytosis*: Release of pore-forming perforin and serine protease granzymes, which enter the cytoplasm and cleave pro-caspases to trigger intrinsic apoptosis.
 2. *Death Receptor Engagement*: Presentation of **Fas Ligand (FasL / CD95L)** on the T-cell membrane, binding to Fas (CD95) death receptors on the cancer cell surface to assemble the death-inducing signaling complex (DISC) and trigger extrinsic apoptosis via caspase-8.
- **Glioblastoma Multiforme (GBM) as an Immunosuppressive Model:**
 - Glioblastoma is an aggressive primary brain tumor that establishes a profound, localized immunosuppressive microenvironment within the central nervous system.
- **Spatial Profiling of Immune Infiltration:**
 - Visualizing tumor slices using multiplex immunofluorescence (DAPI for nuclei, alongside CD3, CD4, and CD8 to identify T-cell populations) reveals distinct spatial distributions:
 - **Perivascular Trapping of T Cells:**
 - In GBM, infiltrating CD3+/CD8+ T cells accumulate within perivascular cuffs immediately surrounding tumor capillaries.
 - Masson's trichrome and immunofluorescence demonstrate excessive perivascular collagen and matrix deposition around the vessel circumference.
 - This dense fibrous layer creates a physical barrier that restricts extravasated lymphocytes to the vessel margins, preventing them from migrating deeper into the malignant parenchymal core.
 - **Radial Distance Infiltration Profiles:**
 - Quantifying immune cell densities across concentric 25-micron radial distance intervals extending outward from the vessel wall (0 μm = vessel perimeter) highlights contrasting behavior:
 - *T-Cell Density*: Drops steeply within the first 25 μm from the vessel edge, falling to near-zero levels in the deep parenchyma.
 - *Macrophage Density*: Macrophages readily penetrate perivascular matrix barriers and migrate deep into the tumor core, showing sustained, high-density distribution hundreds of microns from vessels.

Lecture 12.2: Metastasis

1. Clinical Realities, Timelines, and the Metastatic Cascade

- **The Metastatic Burden in Oncology:**
 - While primary tumors account for roughly 10% of cancer-related mortality, secondary distant metastases drive approximately **90% of all cancer deaths**.
 - Patients often succumb to metastatic relapse years after successful surgical excision of their primary lesion, long after the primary site has ceased to present an immediate clinical problem.
- **The Extended Latency Timeline:**
 - Historically viewed as the final linear step of cancer progression, metastasis is increasingly recognized as a process that can initiate early at the molecular level, even if symptomatic presentation occurs late.
 - In colorectal cancer, the trajectory from the initiating oncogenic somatic mutation to a formal clinical diagnosis of symptomatic metastatic disease can span up to **35 years**.
 - This long latency is common in cancers with non-specific or indolent early symptoms (e.g., vague abdominal discomfort or bloating in colorectal and pancreatic malignancies), resulting in delayed detection.
- **The Sequential Steps of the Metastatic Cascade:**
 1. *Local Invasion:* Malignant cells lose tight cell-cell adhesion, alter their cytoskeletal architecture, and invade surrounding tissue parenchyma and basement membranes.
 2. *Intravasation:* Cancer cells enter systemic conduits—primarily blood microvessels or lymphatic capillaries.
 3. *Circulation Survival:* Circulating tumor cells (CTCs) must withstand high hemodynamic shear stresses, avoid immune clearance (by NK cells and cytotoxic T cells), and overcome specialized detachment-induced apoptosis.
 4. *Arrest and Extravasation:* Cells arrest within microvascular capillary beds of distant organs, cross the endothelial barrier, and enter foreign tissue stroma.
 5. *Micrometastasis and Colonization:* Disseminated cells adapt to the foreign microenvironment, survive in dormancy, and ultimately proliferate into vascularized, clinically detectable macrometastases.
- **The High Cumulative Attrition Rate:**
 - Metastasis is an inefficient biological process with an estimated cumulative attrition rate of approximately **99.98%**.
 - Only a tiny fraction (~0.02%) of cells that leave the primary tumor successfully survive each stage to establish secondary colonies. However, because tumors shed thousands of cells into circulation daily, this minute percentage is sufficient to produce lethal metastases.