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.

Practice Multiple-Choice Questions

Question 1

In clinical epidemiology, how does a diagnosis of morbid obesity (BMI ≥ 40 kg/m^2) alter average life expectancy compared to lean individuals?

A) Life expectancy is extended by approximately 2 years due to preserved subcutaneous fat stores.

B) It has no measurable impact on lifespan once arterial blood pressure is normalized.

C) It reduces life expectancy by approximately 8 years.

D) It decreases life expectancy by exactly 25 years regardless of age at onset.

Question 2

Which histopathological feature distinguishes non-alcoholic steatohepatitis (NASH) from simple steatosis?

A) Total disappearance of all intracellular lipid droplets from the liver

B) Marked hepatocyte ballooning, active lobular inflammation, and cell death

C) Immediate transformation of sinusoidal endothelial cells into bone-forming osteocytes

D) Complete replacement of the portal triad by mature skeletal muscle fibers

Question 3

In long-term clinical cohort studies of patients diagnosed with NASH, what is the single strongest histological predictor of 8-year mortality?

A) The presence and extent of hepatic fibrosis

B) The total volume of gallbladder bile acid storage

C) The absolute diameter of healthy glycogen granules in periportal zone 1

D) The number of circulating reticulocytes in peripheral venous blood

Question 3

Which clinical diagnostic threshold indicates the diagnosis of symptomatic type 1 diabetes on a random plasma glucose evaluation?

A) A fasting blood glucose concentration measuring between 5.5 and 6.9 millimoles per liter

B) A random plasma glucose reading equal to or exceeding 11.1 millimoles per liter

C) An oral glucose tolerance measurement falling between 7.8 and 11.0 millimoles per liter

D) A continuous glucose monitor average tracing remaining below 10.0 millimoles per liter

Question 4

In the genetic architecture of type 1 diabetes, which class of human leukocyte antigen molecules directly presents islet-derived peptides to the primary cytotoxic effector cells executing tissue damage?

A) MHC class II molecules that engage the CD4 coreceptor on naive helper lymphocytes

B) Non-classical CD1d molecules that present microbial glycolipids to natural killer T cells

C) MHC class I molecules that present peptides to autoreactive CD8+ cytotoxic T cells

D) Pre-B cell receptor complexes that display intact insulin to follicular dendritic cells

Question 5

In the classic staging framework of type 1 diabetes, what clinical and metabolic features characterize Stage 2 disease?

A) Complete absence of islet autoantibodies with acute ketoacidosis and muscle wasting

B) Normoglycemia with high titers of a single autoantibody and normal oral glucose tolerance

C) The presence of multiple islet autoantibodies accompanied by dysglycemia in an asymptomatic individual

D) Overt symptomatic hyperglycemia requiring continuous subcutaneous insulin infusion

Answer Key & Detailed Explanations

1. **A** — Rapid physiological responses such as postprandial glucose disposal occur on a minute-to-minute timescale. Covalent modifications like protein phosphorylation modify existing transporter trafficking machinery almost instantaneously, whereas de novo transcription and translation take multiple hours.
2. **B** — Following insulin binding and IRS phosphorylation, PI3K phosphorylates PIP_2 to generate PIP_3. This membrane-bound phosphoinositide recruits both PDK1 and AKT via their pleckstrin homology (PH) domains, allowing AKT phosphorylation and activation.
3. **C** — Muscle contraction uses ATP during cross-bridge cycling and ion pumping, which increases the intracellular AMP:ATP ratio. This activates the energy sensor AMPK, which phosphorylates the Rab-GAPs AS160 (TBC1D4) and TBC1D1, promoting GLUT4 translocation without requiring insulin.
4. **D** — Principal component analysis of human skeletal muscle phosphoproteomes demonstrated that endurance exercise phosphosites largely returned near pre-exercise baseline levels after 3 hours of recovery, whereas resistance exercise and sprint interval training maintained distinct, prolonged post-exercise signaling states.
5. **A** — Genetic studies in Greenland Inuit cohorts identified a common loss-of-function nonsense variant in *TBC1D4* (AS160) that selectively impairs contraction- and insulin-stimulated glucose uptake in skeletal muscle, predisposing carriers to severe postprandial hyperglycemia and Type 2 Diabetes.
6. **B** — Querying InterPro revealed that *C18orf25* shares sequence and structural homology with RNF111, an E3 ubiquitin-protein ligase. This domain profile implicated *C18orf25* in the ubiquitin-proteasome pathway, which regulates muscle protein turnover, remodeling, and mass.
7. **C** — Although GWAS data initially linked the *C18orf25* locus to HbA1c levels, whole-body knockout mice displayed normal glucose excursion curves during glucose tolerance tests and showed no impairment in insulin- or contraction-stimulated 2-deoxyglucose uptake in isolated muscle assays.
8. **D** — When isolated muscle preparations were mounted in organ baths and stimulated with electrical current across different frequencies, *C18orf25* knockout muscles generated significantly less absolute and specific contractile force compared to wild-type controls.
9. **A** — Candidate association studies in human athletic cohorts (including powerlifters and competitive wrestlers from Turkey) revealed that specific alleles of *C18orf25* (*ARK2N*) were present at approximately 40% frequency in elite strength athletes, compared to roughly 20% in sedentary or general population controls.
10. **A** — The *ACTN3* gene encodes α-actinin-3, a structural component of the sarcomeric Z-disc expressed in fast-twitch muscle fibers. Homozygosity for the functional R allele preserves fast-fiber integrity and is heavily enriched in elite sprinters and powerlifters, whereas the homozygous null 577X mutation limits high-velocity, explosive force output.

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 (PrPC)	Pathogenic Scrapie Isoform (PrPSc)
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 ($178\text{N}/129\text{M}$), but causes **fCJD** when paired with a Valine ($178\text{N}/129\text{V}$).

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*.