“Techniques in Cellular and Molecular Neurobiology”

International Master’s Degree in Neuroscience

Lesson 7

DIPARTIMENTO DI SCIENZE DELLA VITA

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Human brain in a dish

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The human brain in a dish: The promise of iPSC-derived neurons

Ricardo Dolmetsch and **Daniel H. Geschwind**

Abstract

Induced pluripotent stem cell-derived neurons from patients promise to fill an important niche between studies in humans and model organisms in deciphering mechanisms and identifying therapeutic avenues for neurologic and psychiatric diseases. Recent work begins to tap this potential, and also highlights challenges that must be overcome for it to be fully realized.

Different models and approaches are complementary and will likely all need to be pursued and integrated to achieve the level of mechanistic understanding needed to develop new therapeutic approaches

Human brain in a dish

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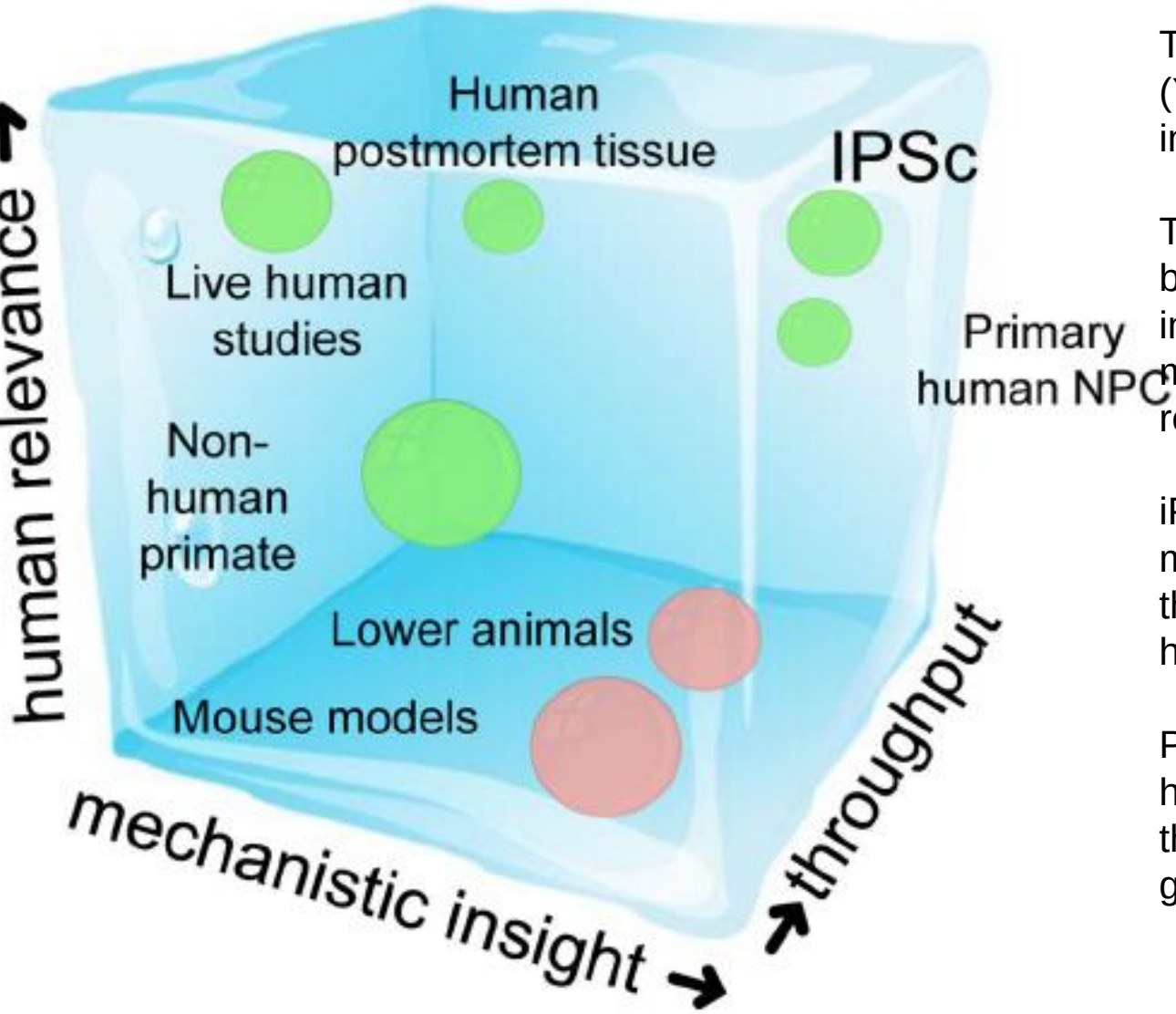

Three axes are depicted, increasing human relevance (Y), increasing molecular or mechanistic insight (X) and increasing throughput (Z).

The primate (most humanoid) systems are labeled in blue and the other vertebrate models are red. Studies in living patients provide high human relevance, but low molecular insights (e.g. functional imaging) and relatively low throughput.

iPSCs promise high human relevance, likely high molecular and mechanistic insight, and relatively low throughput currently, although there is potential for higher throughput with automation and new methods.

Primary human neural cells derived from progenitors harvested from human embryonic brain are higher throughput, but because they may not have all of the genetic background characteristics of specific patients

Primary Neuronal Cultures

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- The complexity of the brain often requires neuroscientists to use a simpler system for experimental manipulations and observations.
- One powerful approach is to generate a primary culture by dissecting nervous system tissue, dissociating it into single cells, and growing those cells *in vitro*.
- Primary cultures make neurons and glia easily accessible to the experimental tools required for techniques like genetic manipulation and time-lapse imaging.
- Furthermore, these cultures represent a highly controllable environment in which to study complex phenomena such as cell-cell interactions

Primary Neuronal Cultures

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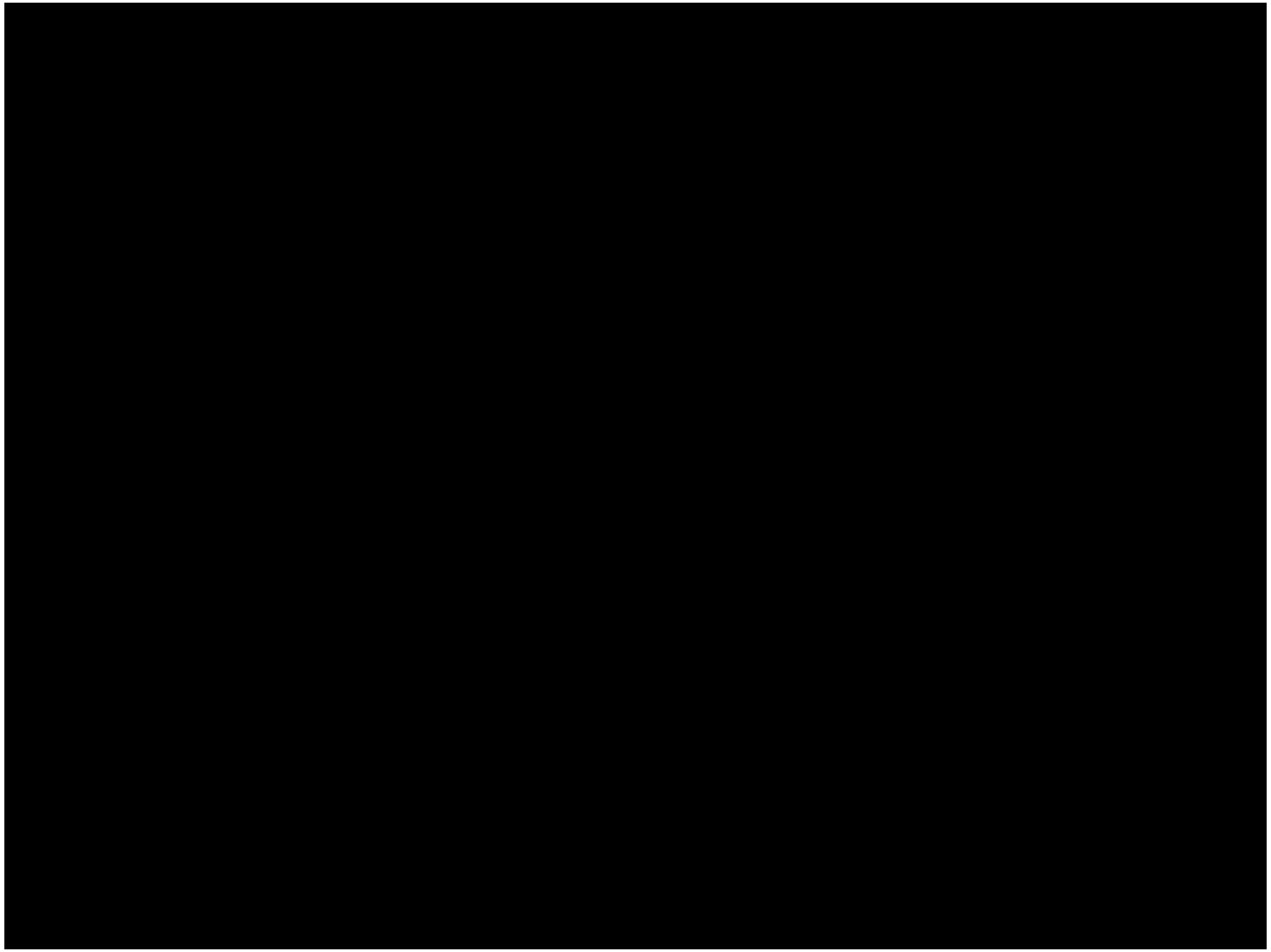

iPSCs to Neurons

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Human induced pluripotent stem cells (hiPSCs) are considered a powerful tool for drug and chemical screening and for the development of new *in vitro* models for toxicity testing, including neurotoxicity.

iPSCs to Neurons

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Video Article

Protocol for the Differentiation of Human Induced Pluripotent Stem Cells into Mixed Cultures of Neurons and Glia for Neurotoxicity Testing

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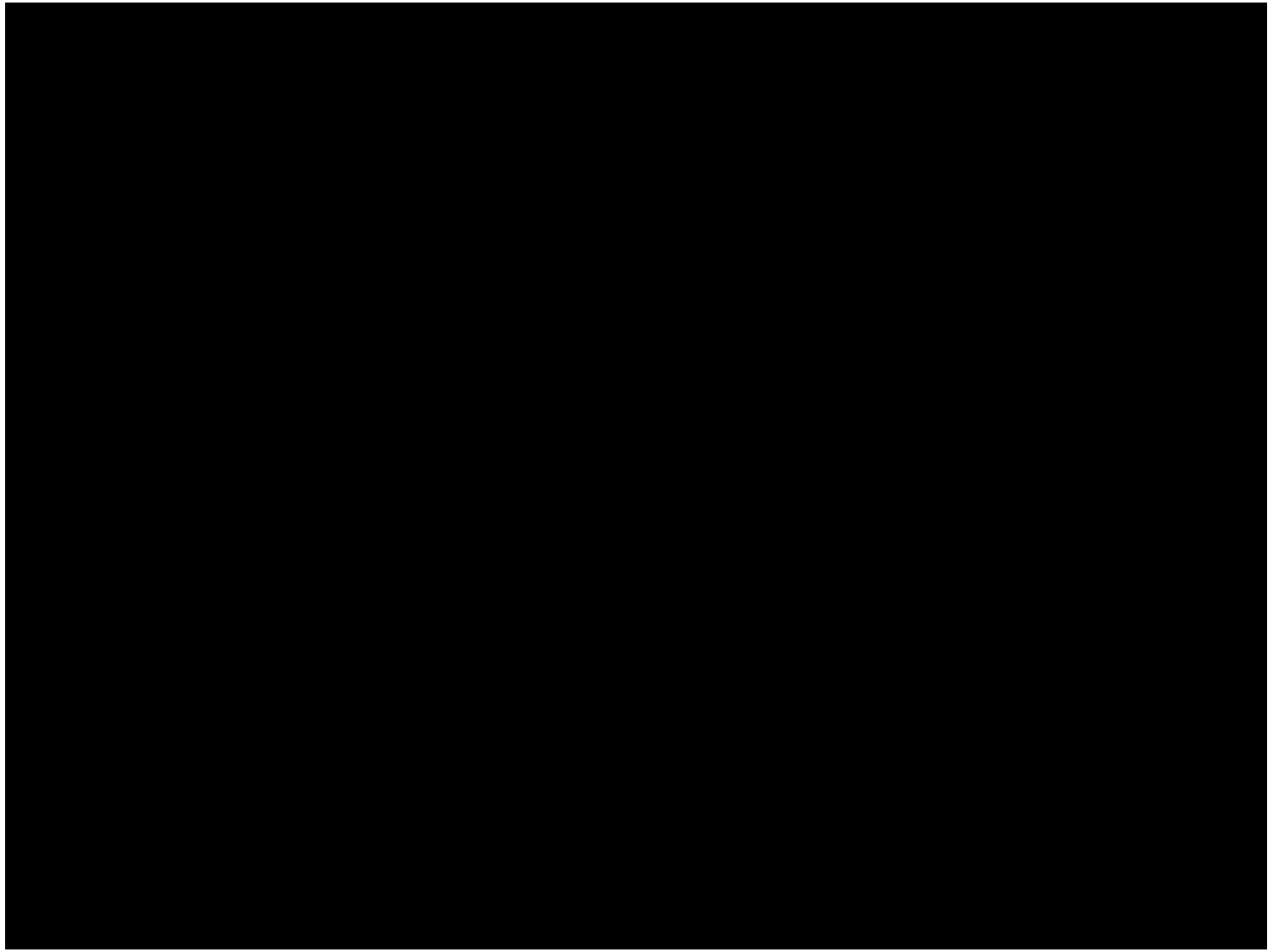

iPSCs to Neurons

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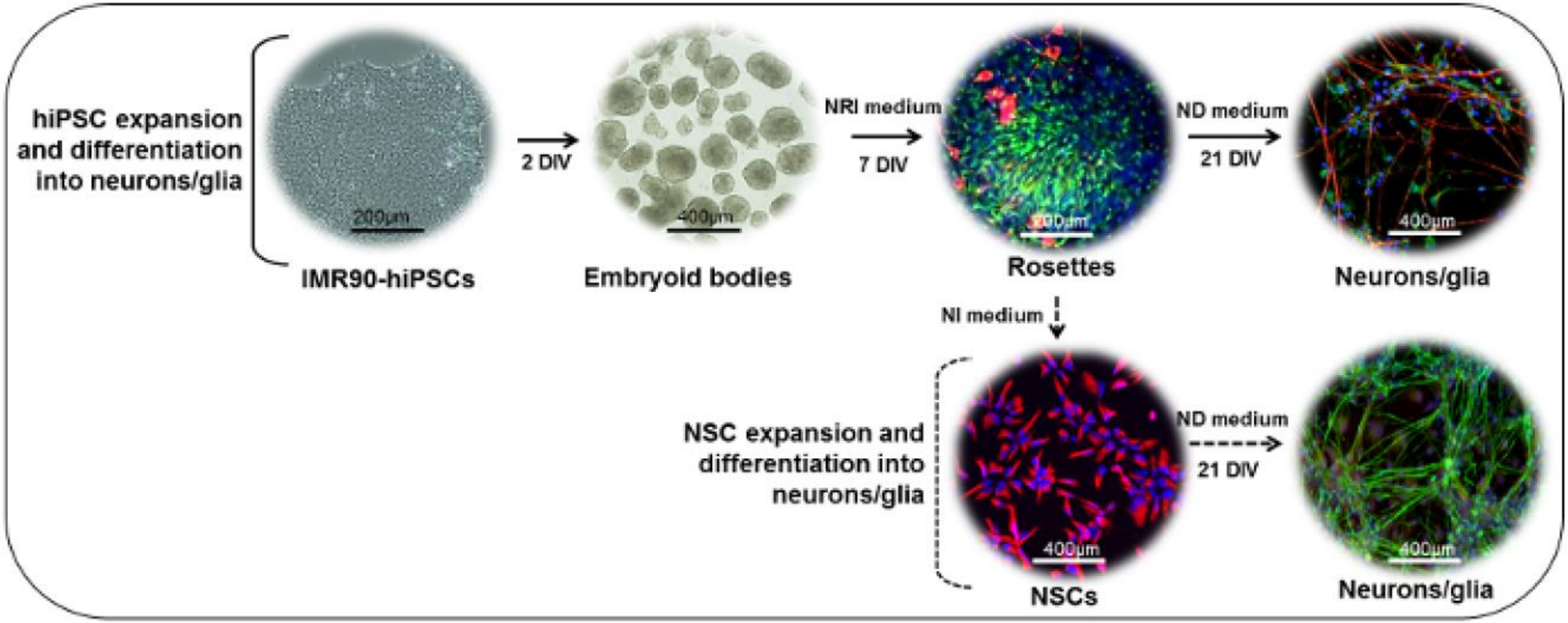

iPSCs to Neurons

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A) HiPSC Differentiation into Mixed Neurons and Glia

1. Generation of embryoid bodies (EBs) (Days 0 → 1)

2. On Day 1, coat the dishes with basement membrane matrix (e.g., matrigel, referred to hereafter as "standard matrix") or any other suitable protein substrate (e.g., laminin).

3. Generation of neuroepithelial aggregates (rosettes) (Days 2 → 7)

4. Rosette dissociation and neuronal differentiation (Days 8 → 28)

B) HiPSC-derived Neural Stem Cell (NSC) Expansion and Differentiation into Mixed Neurons and Glia

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