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DEVELOPMENTAL STAGE DETERMINES FORMATION AND BEHAVIOR OF 3D BRAIN CELL SPHEROIDS

Maiorova O.R. [1], Vsevolodska S.O. [1]

[1] Institute for Problems of Cryobiology and Cryomedicine, NAS of Ukraine

olyamayorova1999@gmail.com

Aim. To determine how the ontogenetic developmental stage of rat brain cells — embryonic (E15) and neonatal (P0) — affects their ability to self-organize into neural spheroids (SF), and to compare their structural-morphological and functional properties during cultivation. **Methods.** Neural cells (NCs) were isolated from the brains of rat embryos (E15) and newborns (P0) using a mechanical–enzymatic dissociation method. Spheroids were obtained using the hanging-drop technique (4×10^3 cells in 20 μL). After 24 hours, the efficiency of SF formation, their diameter, sphericity, and size variability were evaluated. Mechanical stability was determined after repeated pipetting by assessing the proportion of intact SFs and the degree of fragmentation. Functional activity was analyzed after transferring the SFs onto an adhesive substrate, followed by 7 days of observation of cell migration, differentiation, and the formation of intercellular networks. **Results.** Embryonic cells formed SFs significantly more efficiently (82.0%) than neonatal cells (47.9%). Spheroids derived from E15 cells were larger and more spherical (123.5 ± 9.6 μm; circularity 0.87) compared to P0 spheroids (111.0 ± 12.9 μm; circularity 0.75) and exhibited lower size variability. The mechanical stability of embryonic SFs was also higher: about 90% remained intact after testing, whereas among neonatal SFs this value was 51.5%, with nearly half of the spheroids undergoing fragmentation. In addition, embryonic SFs demonstrated greater morphological homogeneity within the population. After transfer to an adhesive surface, both SF types attached but behaved differently. Embryonic SF cells actively migrated, forming a dense confluent layer with signs of neuronal and glial differentiation. Neonatal SF cells showed lower migration, but the migrating cells differentiated and formed stable, organized neuron–glial networks around the spheroids. **Conclusions.** The ontogenetic stage significantly affects the self-organization of neural cells (NCs), as well as the mechanical stability and behavior of the formed spheroids (SFs). Embryonic cells exhibit higher plasticity, enabling the formation of morphologically homogeneous and stable SFs. Despite their lower structural resistance, neonatal SFs retain a high potential for the formation of organized neuron–glial networks. These findings justify the need for a differentiated selection of cell sources depending on the specific objectives of 3D modeling.