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CRYOPRESERVED DISSOCIATED RAT VENTRAL MESENCEPHALIC NEURONS: A SOURCE FOR DOPAMINERGIC NEURON STUDIES IN CULTURE AND COCULTURE

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Primary neuronal cells in culture are an important tool for investigating neuronal mechanisms and responses to pharmaceutical substances or neurotoxicants. Cryopreservation of large batches of dissociated embryonic neurons has proven to be a valid and convenient substitute for fresh dissections as a source of cells for primary neuronal cultures of cortex, hippocampus, striatum and dorsal root ganglia. To increase the variety of brain regions that can be employed in this way we developed a protocol for the cryopreservation and culture of ventral mesencephalic neurons containing significant numbers of dopaminergic neurons. Neurons were thawed and plated onto 96 well plates in neurobasal medium with B27 supplement, no other growth factors were added. After 6 to 12 days the plates were fixed and stained for immunofluorescence using the dendritic marker MAP2 and the dopaminergic marker TH. Evaluations were made using digital image cell counts. At the highest cell density plated dopaminergic neurons numbered 50 cells per 96 well and the cells had excellent morphology. To determine if glial would influence attachment and/or survival, neurons were thawed and plated onto 96 well plates with glial feeder layers derived from cortical, hippocampal or striatal astrocytes. Each increased the numbers of nigral neurons with the best results on cell morphology and density (up to 150 neurons per 96 well) seen using striatal glial cells. Additional experiments were carried out in which cryopreserved ventral mesencephalic neurons from embryonic day 14 were cocultured with cryopreserved striatal neurons from embryonic day 18. Thus, cryopreserved ventral mesencephalic cells are suitable for studying dopaminergic neuronal properties in single cultures and in cocultures with striatum.