Differentiation of Rhesus Embryonic Stem Cells to Neural Progenitors and Neurons.

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Embryonic stem (ES) cells are pluripotent cells capable of differentiating into cell lineages derived from all primary germ layers including neural cells. In this study we describe an efficient method for differentiating rhesus monkey ES cells to neural lineages and the subsequent isolation of an enriched population of Nestin and Musahsi positive neural progenitor (NP) cells. Upon differentiation, these cells exhibit electrophysiological characteristics resembling cultured primary neurons. Embryoid bodies (EBs) were formed in ES growth medium supplemented with 50 % MEDII. After 7 days in suspension culture, EBs were transferred to adherent culture and either differentiated in serum containing medium or expanded in serum free medium. Immunocytochemistry on differentiating cells derived from EBs revealed large networks of MAP-2 and NF200 positive neurons. DAPI staining showed that the center of the MEDII treated EBs was filled with rosettes. NPs isolated from adherent EB cultures expanded in serum free medium were passaged and maintained in an undifferentiated state by culture in serum free N2 with 50 % MEDII and bFGF. Differentiating neurons derived from NPs fired action potentials in response to depolarizing current injection, and expressed functional ionotropic receptors for the neurotransmitters glutamate and gamma-aminobutyric acid (GABA). NPs derived in this way could serve as models for cellular replacement therapy in primate models of neurodegenerative disease, a source of neural cells for toxicity and drug testing, and as a model of the developing primate nervous system.