

ABSTRACT 427.16 SFN2003

CRYOPRESERVED MOUSE CORTICAL AND STRIATAL NEURONS IN PRIMARY CELL CULTURE. W.A. Staines1*; B. Tinner-Staines2; K. Vaillancourt2; D. Gray3; A. Krantis1. 1Dept Cellular & Molecular Medicine, Univ Ottawa, Ottawa, ON, Canada. 2QBMCellScience, Ottawa, ON, Canada. 3Cancer Therapeutics, Ottawa Health Research Institute, Univ Ottawa, Ottawa, ON, Canada

Introduction

Previously, we have shown that cryopreserved rat embryonic cortical neuronal cells can be thawed and grown in primary culture with complete preservation of functional characteristics. We now demonstrate cryopreservation and subsequent culture of mouse embryonic cortical and striatal neuronal cells. Single dissociated cultures of either cryopreserved cortical or striatal cells were cultured in 24 or 96 well plates for 7 to 21 days. Similarly, cocultures containing both cortical and striatal cells were studied.

Dissociated primary cell culture is the most widely used *in vitro* system in neurobiology. However, the financial cost and resources associated with obtaining viable neuronal cultures at each research site can be high and in many instances, prohibitive. There are also many scheduling difficulties and transfer of primary neuronal cell cultures between laboratories results in significant cell loss.

The ability to cryopreserve primary neurons would maximise the yield of dissociated cells/animal, decrease the number of animals required for each study, reduce labour costs, allow transfer of material between collaborating laboratories and minimise any time delay in experimentation. We show here that mouse neuronal cells from different brain regions can be cryopreserved, thawed and studied in primary culture and coculture.

Methods

Culture

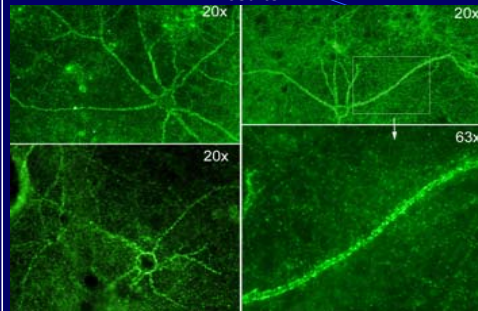
Vials of cryopreserved mouse primary cortical or primary striatal neurons (QBMCellScience) were removed from liquid nitrogen vapour phase storage and thawed for 2.5 min at 37°C. 9 ml of Neurobasal medium supplemented with B27 was added slowly and cells were plated on coated coverslips in 24 well plates or on coated 96 well plates at densities of from 400 to 40 K. After 2 hrs they were changed to fresh medium and then not again until 7 days *in vitro* (1/2 media changes every 3 to 4 days thereafter). These were fixed in 4% paraformaldehyde containing picric acid for 1 hour, and washed with sodium phosphate buffer (PBS).

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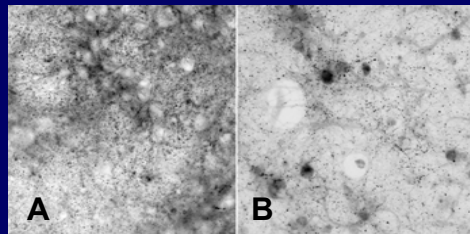
Cultures were incubated with primary antibodies for 2 hours at 37 °C and with secondary antibodies for 45 minutes at 37 °C.

Antibody	Raised in	Source
Green Fluorescent Protein	Monoclonal (Mouse)	Sigma
Synapsin	Polyclonal (Rabbit)	IF
Vesicular GABA Transporter (vGat)	Polyclonal (Guinea Pig)	Chemicon
Vesicular Glutamate Transporter (vGlut1)	Monoclonal (Mouse)	Synaptic Syst

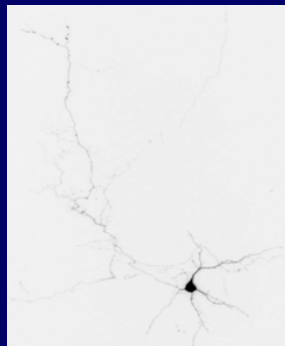
Results



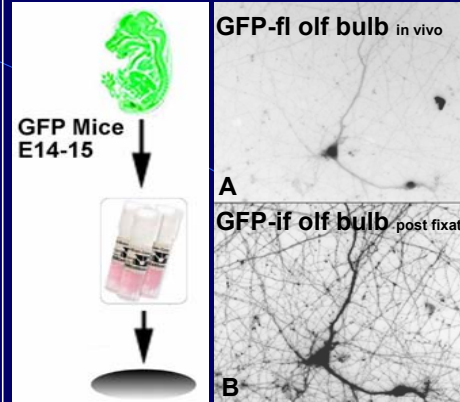
Synapsin around soma and dendrites of sparsely plated cryopreserved cortical CD1 mouse primary neurons, 21 days in vitro



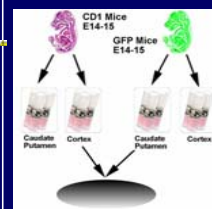
Synapsin immunoreactivity representing total synaptic density in the neuropil at the center of a 21 day culture of CD1 mouse cortical primary neurons (A) compared to vGAT terminal density in a similar field (B)



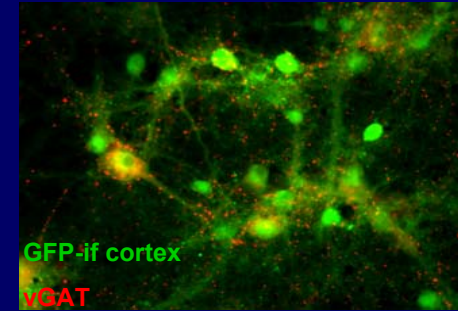
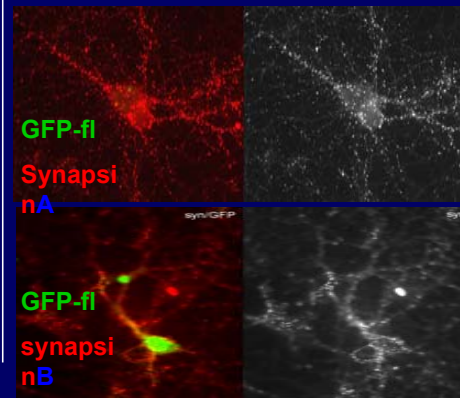
Cryopreserved primary CD1 mouse cortical cell transfected with eGFP using TransMessenger Transfection Reagent (Qiagen) at 7 days in vitro



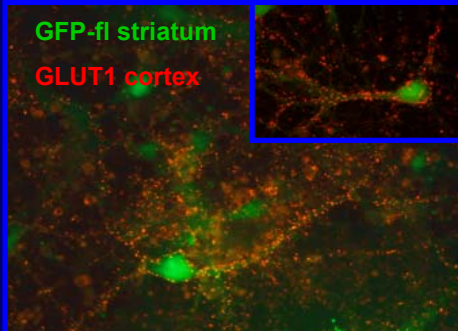
Cryopreserved primary olfactory bulb neurons from GFP mouse showing endogenous GFP (A) and enhanced GFP by anti-GFP (B)



Cryopreserved CD1 mice cortical primary neurons cocultured with cryopreserved GFP mice primary striatal neurons showing Synapsin around soma and dendrites of cortical non GFP neurons(A) and striatal GFP neurons(B)



Cortical VGAT positive nerve terminals making contact with the soma and dendrites of cryopreserved GFP primary striatal neurons in corticostriatal cocultures, 21 days in vitro



Cortical GLUT1 positive nerve terminals appear to contact extensively the soma and dendrites of GFPfl striatal neurons in corticostriatal cocultures, 21 days in vitro

Conclusion

Results show that both primary mouse cortical neurons and primary mouse striatal neurons can be cryopreserved and stored frozen for subsequent cell culture study. Furthermore, cocultures of dissociated mouse cortical neurons and striatal neurons survive over a wide range of cell plating density ratios and display many of the neurochemical characteristics of neurons in tissue section. This represents a major advance for neuronal cell culture-based research.