

ABSTRACT 427.14 SFN2003

CRYOPRESERVED HIPPOCAMPAL NEURONS IN PRIMARY CELL CULTURE. A. Krantis1\*; Q. Di Ilio2; S. van den Hoek2; B. Tinner 2; W.A. Staines1. 1Dept of Cellular & Molec Med, Uni of Ottawa, Ottawa, ON, Canada. 2QBMCellScience, Ottawa, ON, Canada

**Abstract**

Previously, we have shown that rat embryonic cortical neuronal cells can be cryopreserved and subsequently thawed and grown in primary culture with complete preservation of functional characteristics. We now present evidence for the successful cryopreservation and culture of hippocampal neuronal cells. Dissociated cultures of cryopreserved hippocampal neurons were incubated in 24 or 96 well plate cultures for from 7 to 21 days *in vitro*. They were then fixed and characterized neurochemically by immunohistochemical techniques employing antibodies against GABA and glutamate vesicular transporters, GABA and glutamate receptors and structural marker proteins. Results show that both primary hippocampal neurons can be cryopreserved and stored frozen for subsequent cell culture study.

**Introduction**

Dissociated primary cell cultures are widely used *in vitro* system in neurobiology and are often the most authoritative *in vitro* model for molecular and physiological experiments. These can be time consuming and capricious for all but the most experienced laboratory personnel. We show that simple culture protocols using commercially available cryopreserved hippocampal cells provide a practical alternative to live embryo dissections for primary culture of hippocampal neurons.

The ability to cryopreserve primary neurons would maximize the yield of dissociated cells/animal, decrease the number of animals required for each study, reduce labor costs, allow transfer of material between collaborating laboratories and minimize any time delay in experimentation. This represents the focus of the rationale for our work in developing a procedure for dissociating neurons from the brain and cryopreserving these cells for subsequent use.

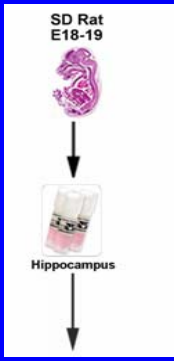
We have successfully cryopreserved rat embryonic hippocampal neuronal cells which can be thawed and grown in primary culture.

**Methods**

Evaluation of these primary cell cultures for their s...

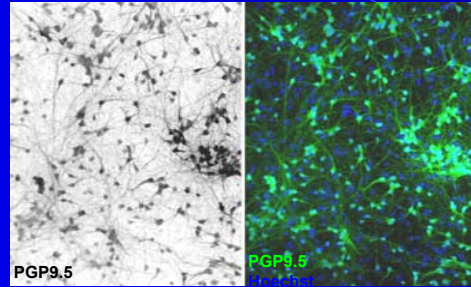
Vials of cryopreserved rat primary hippocampal neurons E19 (QBMCellScience) were removed from liquid nitrogen vapor phase storage and thawed for 2.5 min at 37° C. 9 ml of Neurobasal medium supplemented with B27 was added slowly and cells were plated on coated coverslips in 24 well plates or on coated 96 well plates at densities of from 400 to 40 K. After 4 hrs they were changed to fresh medium and then again at 4 days *in vitro* (1/2 media changes every 4 days thereafter).

Cryopreserved rat hippocampal cultures were fixed in 4% paraformaldehyde containing picric acid for 1 hour, and washed with sodium phosphate buffer (PBS). Cultures were incubated with primary antibodies for 2 hours at 37 °C and with secondary antibodies for 45 minutes at 37 °C.

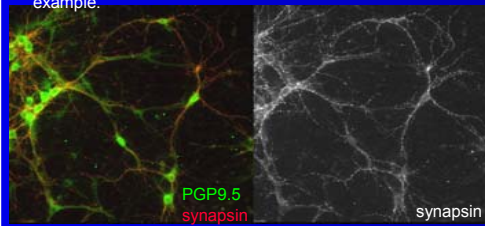


GABA	Rabbit	Chemicon
PGP 9.5	Rabbit	Chemicon
Synapsin	Monoclonal (Mouse)	Chemicon
Vesicular GABA Transporter (vGAT)	Guinea Pig	Chemicon
Vesicular GABA Transporter (vGAT)	Rabbit	Affinity
Vesicular Glutamate Transporter (vGLUT1)	Guinea Pig	Chemicon

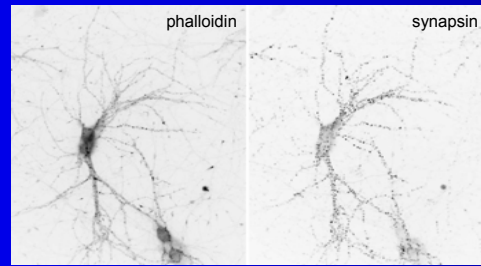
**Results**



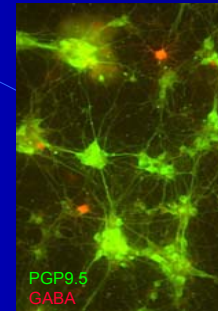
Cryopreserved hippocampal neurons showing positive PGP 9.5 immunolabeling together with Hoechst staining of nuclei. Glial cells are present in this serum supplemented example.



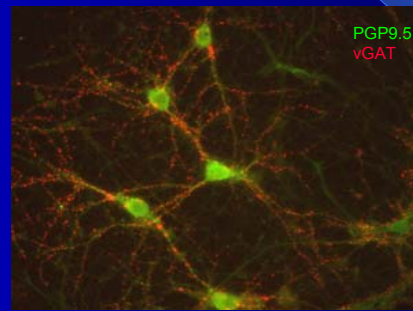
Cryopreserved hippocampal neurons showing positive PGP 9.5 immunolabeling have extensive apparent contacts with synapsin positive varicosities.



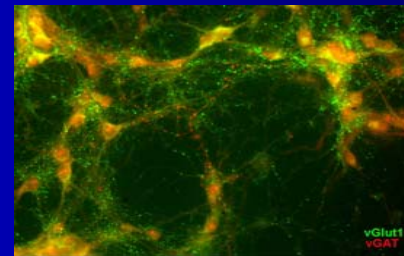
Cryopreserved primary hippocampal culture showing synapsin immunolabelling of nerve terminals together with phalloidin to label dendritic spine head actin, 19 days *in vivo*



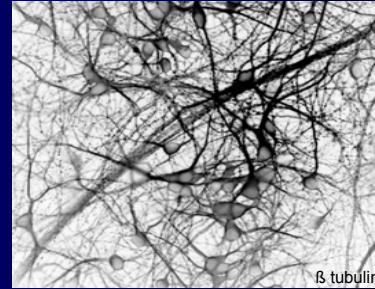
Cryopreserved hippocampal primary cells thawed and cultured showing neurons positive for anti PGP9.5 and a subpopulation which are positive for GABA



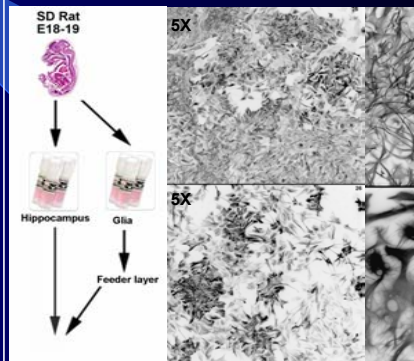
Cryopreserved hippocampal cultures showing vGAT positive terminals making contact with soma and dendrites of PGP 9.5 positive neurons



Hippocampal cryopreserved neurons showing double labeling for GABAergic and glutamatergic using rabbit anti vGAT and GP anti vGLUT. Double labeled varicosities are not apparent and glutamatergic varicosities are by far the more abundant.



Cryopreserved hippocampal primary neurons showing positive immunoreactivity for beta tubulin



**Conclusion**

Given that primary hippocampal neurons after cryopres show the same characteristics as freshly prepared neurons, we propose that cryopreserved hippocampal cells offer advantages over freshly dissociated hippocampal cells: no animal handling is required, and there is no need for the time consuming preparation of neurons. Since the cryopreserved hippocampal cells are pooled from 80 to 90 embryos a larger reservoir of homogenous cell cultures is supplied. Thus, using the standardized cell batch results in a higher degree of comparability even between different laboratories and for long term series of experiments.