

ABSTRACT 427.15 SFN2003

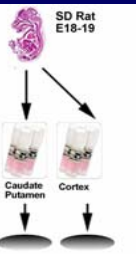
COCULTURE OF CRYOPRESERVED RAT PRIMARY CORTICAL AND STRIATAL NEURONS. B. Tinner-Saines<sup>1\*</sup>; W.A. Staines<sup>2</sup>; N. Cote<sup>1</sup>; S. van den Hoek<sup>1</sup>; A. Krantis<sup>2</sup>. 1QBMCellScience, Ottawa, ON, Canada, 2Cellular and Molecular Medicine, University of Ottawa, Ottawa, ON, Canada

Most primary neuronal cell cultures use neurons from a single brain region. This does not adequately model function as deafferented neurons show biochemical and physiological abnormalities that limit scientific study and preclude drugs screening. A few labs employ neuronal cocultures in which brain components are reassembled to more closely mimic the native state. We demonstrate that this can be accomplished easily using coculture of cryopreserved primary neural cultures isolated from different brain regions and embryonic developmental stages. Previously, we have shown that rat embryonic cortical neuronal cells can be cryopreserved and then thawed and grown in primary culture with complete preservation of functional characteristics. Cryopreserved primary cortical and striatal neurons were thawed, plated in tandem and incubated in 24 or 96 well plates cultures for from 7 to 35 days in vitro. Cryopreserved mesencephalic dopamine neurons were added to some cocultures at 7 day in vitro. Cultures were characterized employing antibodies to synaptic markers, receptor and second messenger proteins and structural marker proteins.

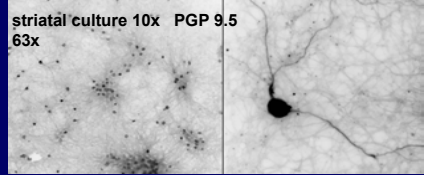
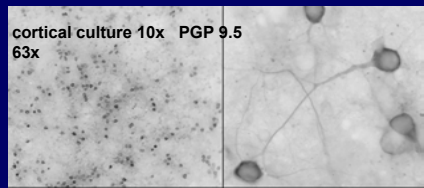
**Methods**

**CULTURE** Vials of cryopreserved rat primary cortical or primary striatal 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 not again until 4 days in vitro (1/2 media changes every 3 to 4 days thereafter).

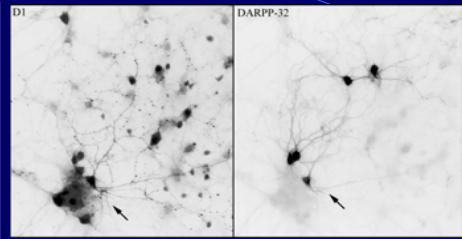
**IMMUNOHISTOCHEMISTRY** At 7 to 35 days in vitro (DIV) cultures were fixed in 4% paraformaldehyde containing picric acid for 1 hour, and washed with sodium phosphate buffer (PBS). Cultures were incubated with primary antibodies for 2 hours at 37 °C and with secondary antibodies for 45 minutes at 37 °C



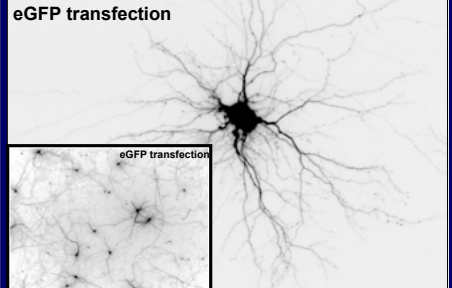
DARPP-32	Rabbit	Chemicon
PGP 9.5	Rabbit	Chemicon
Synapsin	Monoclonal (Mouse)	Chemicon
Vesicular GABA Transporter (vGAT)	Guinea Pig	Chemicon
Vesicular Glutamate Transporter (vGLUT1)	Guinea Pig	Chemicon



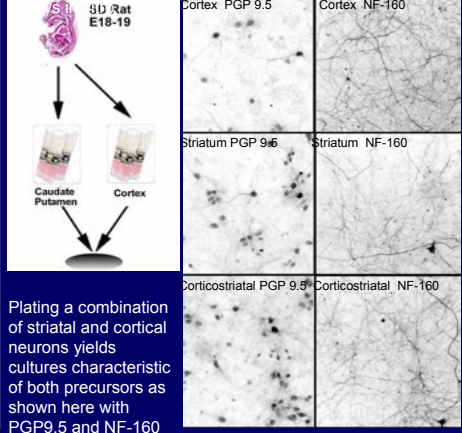
Single primary cortical and striatal cultures 24 div stained with antisera against neuronal marker PGP 9.5



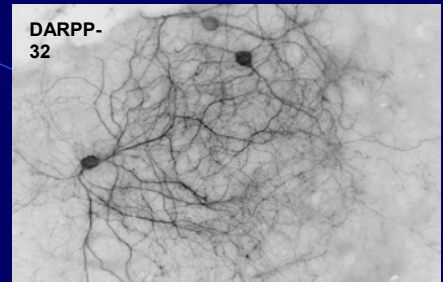
Colocalised D1 and DARPP-32 positive neurons in cryopreserved primary striatal neurons



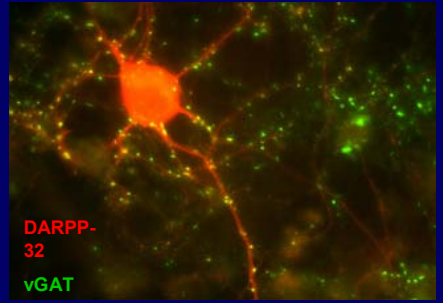
Cryopreserved primary cortical neurons transfected (8 to 10%) with eGFP Transmessenger Reagent (Qiagen) 7



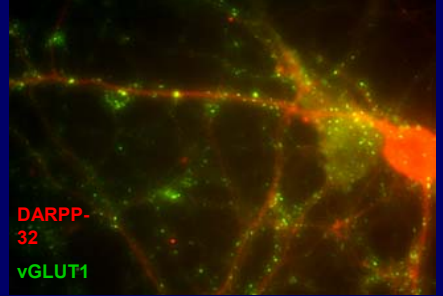
Plating a combination of striatal and cortical neurons yields cultures characteristic of both precursors as shown here with PGP9.5 and NF-160 staining



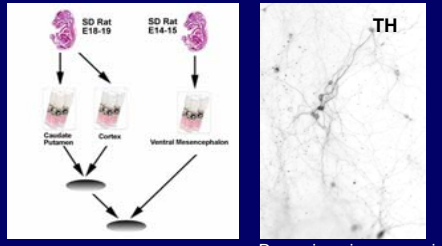
DARPP-32 stained neurons in a corticostriatal coculture 35



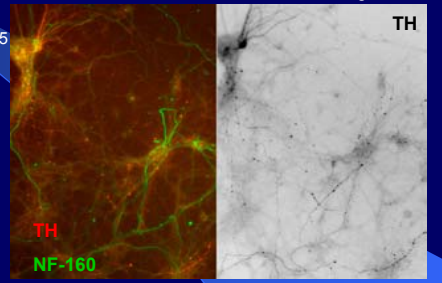
Cryopreserved primary cortical and striatal neurons thawed and cocultured for 21 days vGAT positive nerve terminals making contact with soma and dendrites on a



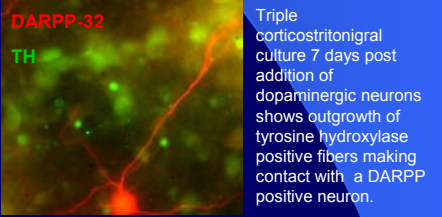
Corticostriatal coculture, 21 div, vGLUT1 positive nerve terminals contact a DARPP positive neuron. Note DARPP negative neuron to its right has greater somatic contact



Dopaminergic neurons in corticostriatal coculture



Triple culture of cortex, striatum and ventral mesencephalon



Triple corticostriatal culture 7 days post addition of dopaminergic neurons shows outgrowth of tyrosine hydroxylase positive fibers making contact with a DARPP positive neuron.

**Conclusion**

Cocultures of cortical and striatal neurons from cryopreserved E19 rat primary cells thrive and display a combination of characteristics expected of these cells in combination.

Some evidence for cross innervation – glutamatergic innervation of medium sized DARPP positive neurons – is seen. (See also 427.16)

Cryopreserved E14 rat primary mesencephalic cells plated onto corticostriatal cocultures thrive and display dopaminergic neurons with active outgrowth of tyrosine hydroxylase stained axonal processes.

Corticostriatal and corticostriatalnigral dissociate cultures appear viable models for examining questions of basal ganglia function and toxicology.