Cryopreserved Neurons: Made in Canada

A major advance for modeling brain function

Anthony Krantis, PhD
May 2011
Frustration can drive invention …need to

1) Maximize use of isolated neurons
2) Avoid dependence upon ordering/delivery/facility housing of animals
3) Avoid inter-experiment variability …cannot QC freshly prepared cells
5) Provide collaborators with neurons from same batch
6) Archive neurons for follow-on studies

Why cant we simply freeze the isolated neurons?
**Patent:** Technology for cryopreservation of primary animal neuronal and non-neuronal cells as Ready To Use products for R&D and regulatory applications
Product Concept to Delivery

- Design Development
- Manufacturing & Testing
- Product Specifications
- Product Assembly & QC testing

R&D (65%)
• World’s leading supplier for cryopreserved rodent neuronal & non-neuronal cells
• Quality validated: 106 publications since 2003
• Ideal for R&D, diagnostic or screening applications

Distributed by LONZA Inc.
Ready-To-Use Rat Brain Microglia

Overcomes difficulty in reliably obtaining reproducible, high yield, pure, viable microglia

Studied for their role in Alzheimer’s, HIV Encephalitis and MS
A ready supply of batch tested, high quality animal cells for primary culture offers......

- Major step for neuro- & biomedical R&D
- Revolutionizes R&D as did ‘antibodies’ & molecular ‘kits’
- Opportunity for non-expert users
- Streamlined workflow
- Large supplies of standardized primary cells

qbmcellscience.com
Ready-To-Use Brain Neurons

“This is a major advance for cell-culture based research”
Biotechnology Magazine
“The aspect of your cryopreserved cortical cells is excellent and 'biological response' looks very robust.”

Abbott GmbH

Rat Cortical Neurons - 21 days
Anti-VGAT red (synaptic)
Anti-Synapsin green
“Neurons in a ready-to-use form makes these cells a valuable tool for CNS research and drug discovery”

Cellomics
Soc Biomol Screening

Cerebellar GABA neurons: green

Rat Cerebellar Neurons
Day 8
Your DRG cells 19 days. Patch clamped, Jan 22/08. They were excitable and got nice ephys.
(NRC, New Brunswick)
Frozen, *Ready-to-Use* Neurons: An enabling technology

1. **hNT Cells** (human neuronal cell line)
   - 6 wks incubation with retinoic acid

2. Purification of Cells

3. Culture
   - Survival time > 60 days but Network activity inconsistent

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1. **QBM Neuronal cells**
   - Thaw & Culture

   - Survival time on MEA
   - > 80 days
   - [523 days]
Neurochips allow rapid and sensitive screening

<table>
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<th>Inhibition</th>
<th>IC$_{50}$</th>
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<tr>
<td>TTX</td>
<td>1.1 nM</td>
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<tr>
<td>GABA</td>
<td>1 μM</td>
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<tr>
<td>Magnesium</td>
<td>161 μM</td>
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<tr>
<td>APV</td>
<td>18 μM</td>
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<th>Biphasic</th>
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<tr>
<td>NMDA</td>
<td>excitatory $&lt; 1$ μM</td>
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<tr>
<td></td>
<td>inhibitory $&gt; 1$ μM, IC$_{50}$ $= 1.8$ μM</td>
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<tr>
<td>Glutamate</td>
<td>excitatory $&lt; 5$ μM</td>
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<tr>
<td></td>
<td>inhibitory $&gt; 5$ μM, IC$_{50}$ $= 10$ μM</td>
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Cerebrospinal Fluid of Brain Trauma Patients Inhibits in vitro Neuronal Network Function via NMDA Receptors

‘Fingerprinting patients’
Early diagnosis
better patient management and outcomes

Rat cortical neurons - neurochip
Innovation: Reconstructing the brain in a dish

Stroke  Parkinson’s  Alzheimer’s Disease  Brain Cell Interactions

neurochip
The future for R&D, diagnostics and cell biology

*Easy-To-Use, advanced cell interrogation/screening platforms*

- Research tools
- Biosensors
- Screening assays
- Diagnostic tools
Advanced Neurochips: Made in Canada

Synaptic communication

Patch-clamp through micro-hole

Cell placement and guided growth

Micro-fluidic chambers

Multi channel voltage controller and current amplifier

Fibre optic probes

Chemical sensors

G. Mealing

patent applications:
US601,568,220 PCT/CA05/00682, May 2005
EPO 0574136.1-1521-CA2005000682, Dec 2006
Modeling Brain Circuits in Culture using Cryopreserved neuronal cells from different brain regions
Cryopreserved neurons cultured on MEA with superimposed electrode activity

Neurons initially plated with a barrier separating cortical from striatal.

After barrier removed axons crossed the unplated zone

Cortical activity correlated with firing response in striatal neurons.

Severing connections (across unplated zone) preserved Cortical firing but abolished Striatal activity.

Frontiers in Neuroanatomy-Review: Nov 2010
Marianela Garcia-Munoz, Luis Carrillo-Reid and Gordon W. Arbuthnott*
We can now reconstruct brain circuits in a dish

Capturing and understanding the information generated in that dish, is the next innovation.
Thank you

This presentation included

1. Neuronal modeling data provided by the Global Network for Brain Reconstruction

2. MEA data from the Neurochip Laboratory, uDusseldorf

3. Data provided by Cellomics; AMAXA; Cellectricon; MultiChannel Systems; Qiagen GmbH; Astra Zeneca Inc; Phosphosolutions; Merck Inc; National Research Council (Canada)
Ottawa ...
Ideal place for freezing cells...