

# europaean Life Science No2

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# Frozen neurons change research workflow

By Anders Lindgren

In today's world, the cost of research, both in time and infrastructure is high and the unpredictability of animal based research is ever present. How do you protect against this? How do you streamline your research workflow?

**CELL CULTURE** This is what faced Dr. Anthony Krantis and Dr. William Staines, both professors at the Centre for Research in Biopharmaceuticals and Biotechnology, University of Ottawa who co-founded QBM Cell Science Inc. in 2000 based on their discovery of how to cryopreserve adult rat neurons. First accomplished with rat enteric neurons, the know-how was quickly applied to cryopreserving brain and spinal cord neurons. When thawed and cultured the neurons display normal morphology (Fig. 1) and function in long-term culture. QBM has thawed cells archived for 6 years and found them to display normal activity in culture.

The impetus for the discovery was the prohibitive tedium, caprice and inefficiency of obtaining primary neuronal cells. They also wanted to be able to share neuronal cells with collaborators. In contrast to fresh neurons, cryopreserved neuronal cells can be shipped anywhere. They can also be archived, so researchers can re-visit past experiments – on the same batch of cells. This represents a significant advantage for reproducibility across time. Another consideration was the desire to reduce animal usage, which carries immense benefits for laboratory and institutional facilities. For researchers/institutions who previously were limited to cell line based culture studies, this allows them to undertake primary neuronal cell studies.

They commercialised the patented technology and then partnered with QIAGEN to become the first commercial source for cryopreserved rodent brain and spinal cord neurons. Product offerings include cells from multiple brain regions of rat and mouse. A large part of QBM's activities is custom preparation of neuronal cells from valuable transgenic mice and rodent animal models for industry and institutional research groups. QBM in effect serves as a 'core primary cell facility'.

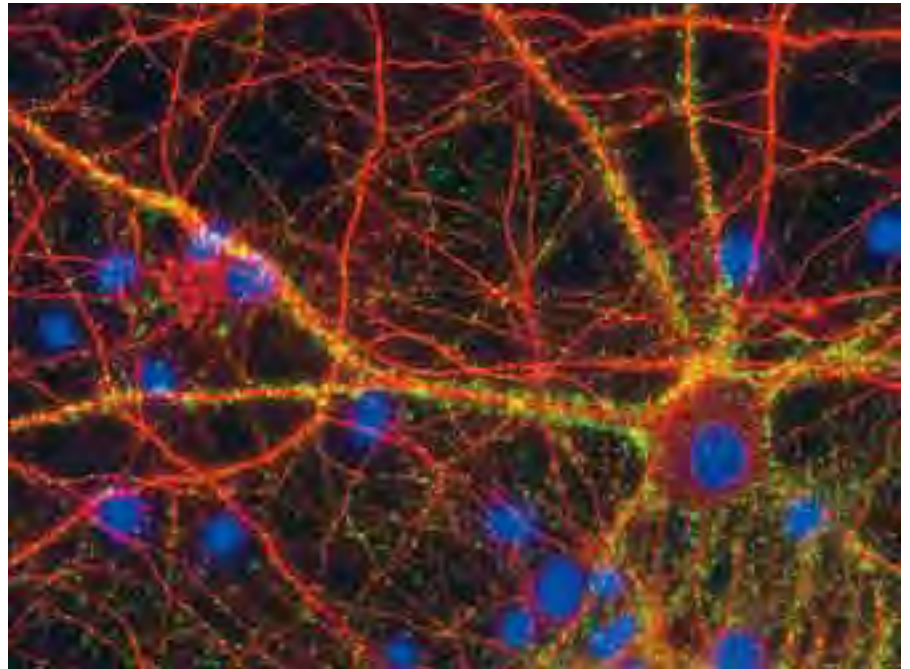


Fig 1. Cryopreserved rat cortical neuronal cells cultured 21 days.

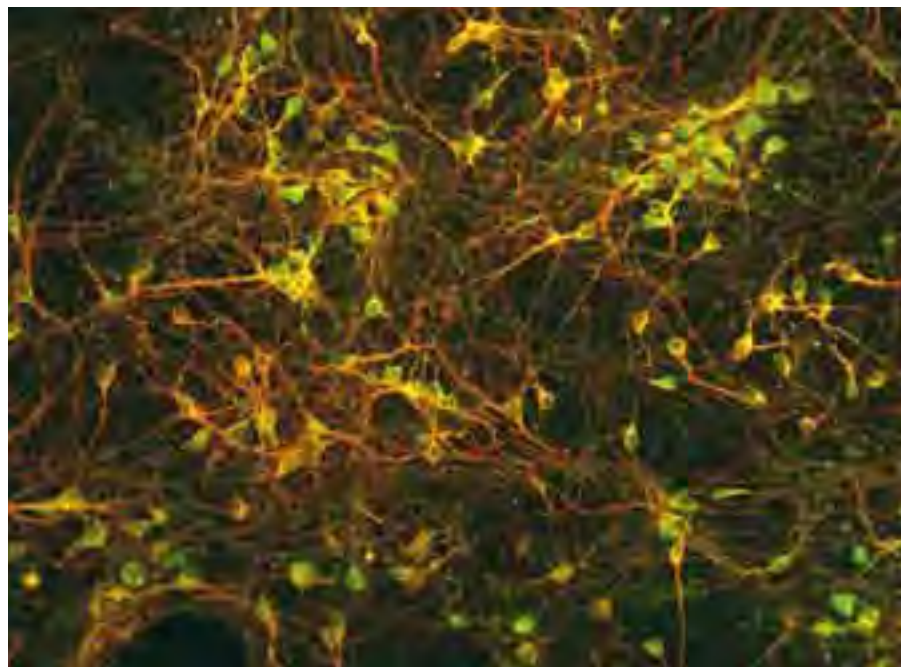


Fig 2. Cryopreserved rat brain neurons in culture – display.

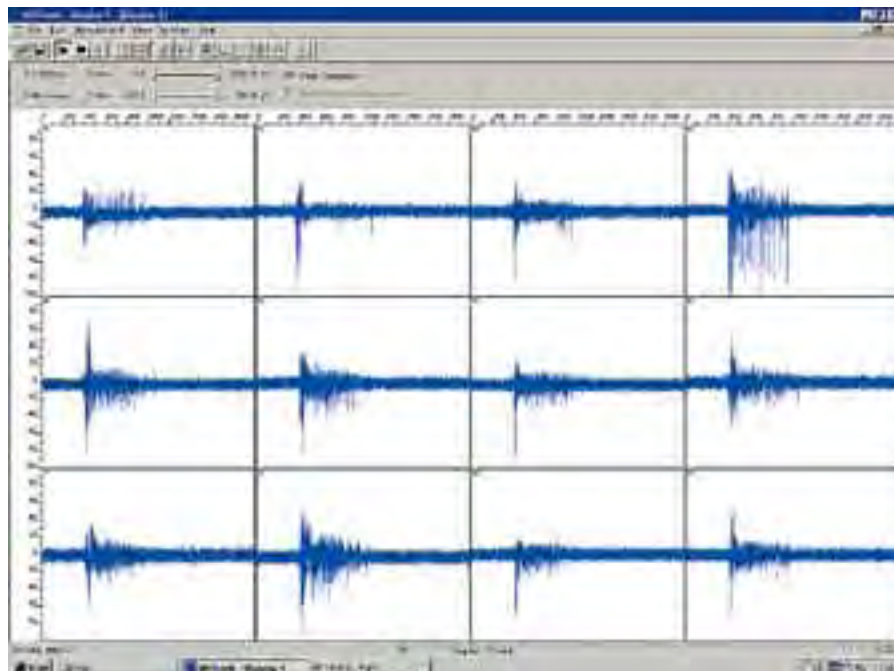


Fig 3. Sample neurochip recording of cryopreserved rat cortical.

Shipped frozen, the cells can be thawed and used, when and how the researcher/groups/regulatory agencies and industry want. This eliminates the inflexibility and cost of animal handling necessary to obtain neuronal cells. Each batch of cryopreserved cells undergoes QC; samples of cells are placed in dry ice shippers for 24 hours, and then cultured. The progress of the cells are chronicled for seven days and subjected to a battery of tests compared to a reference batch. This ensures a record of exactly what each batch will look like for the client. QBM also provides extensive technical support.

Cryopreserved neuronal cells display normal electrophysiology, receptor expression, pharmacology and are ideal for screening pharmacological substances or clinical samples, as demonstrated by the Neurochip laboratory, Univ. Düsseldorf who routinely run cryopreserved neuron-based neurochips for >400days (Fig. 2 and 3). Treated with H<sub>2</sub>O<sub>2</sub>, Kainic acid or Glutamic acid, up to 100µM, the neuronal cells also show normal sensitivity to oxidation or toxicant induced neurotoxicity.

#### A robust test model

For researchers studying neurological disease, cryopreserved neurons from different developmental ages offers the ideal approach to culture studies in which brain components are re-assembled to more closely mimic the native state. This represents a robust test model for acute/chronic drug exposure studies, reproducing the

cellular interactions that occur in vivo, including morphogenesis and synaptogenesis. Another advantage is the possibility for studying early markers of neurotoxicity.

Researchers at the Okinawa Inst. of Science and Technology (OIST) have shown the flexibility and efficiency of this approach. Employing QBM cryopreserved striatal neurons from different developmental ages in co-culture, allowed expression of cholinergic interneurons, which are a critical driver neuron in the striatum (Fig. 4). This sets the stage to model striatal circuitry and for developing multineuronal cultures where cryopreserved ventral mesencephalon neurons (dopaminergic neurons) are added to the co-culture providing the complete circuitry for studying Parkinson's Disease.

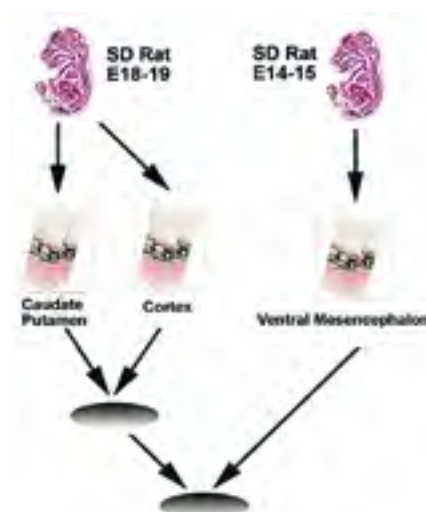


Fig 4. Using cryopreserved neurons for brain modelling studies.

## “The impetus for the discovery was the prohibitive tedium, caprice and inefficiency of obtaining primary neuronal cells”

Cryopreserved striatal and cortical cells which represent the brain regions related to Huntington's Disease, or cryopreserved hippocampal cells associated with early stage disturbances in Alzheimer's, can be easily co-cultured and represent an advance in the study of brain disease and drug screening.

QBM is now applying its cryopreservation technology to other mammalian systems where primary culture is an important R&D approach. QBM recently introduced cryopreserved neonatal rat ventricular cardiomyocytes, which are one of the most widely used cardiac cells for the study of cardiac function in health and disease. When thawed, the cardiomyocytes thrive in long-term culture displaying normal morphology and cyclical beating, with typical gap-junctional activity and pharmacology.

If one had to consider how cryopreserved primary cells shipped 'on demand' might impact the research vista, we need only recall when anti-bodies were available to only a few labs that could produce them. Now these are a standard research tool with wide commercial availability. Indeed, anti-bodies revolutionised the neurosciences. ❖

## Biotech research agency

The National Research Council is the premiere biotechnology research agency of the Canadian federal government. The NRC Biotechnology Program was established in 1983 under the guiding principles of the National Biotechnology Strategy.

Five partner institutes deliver the NRC Biotechnology Program with support from NRC Industrial Research Assistance Program (NRC-IRAP) and NRC Canada Institute for Scientific and Technical Information (NRC-CISTI) for technology transfer and knowledge dissemination. ❖