CASE STUDY

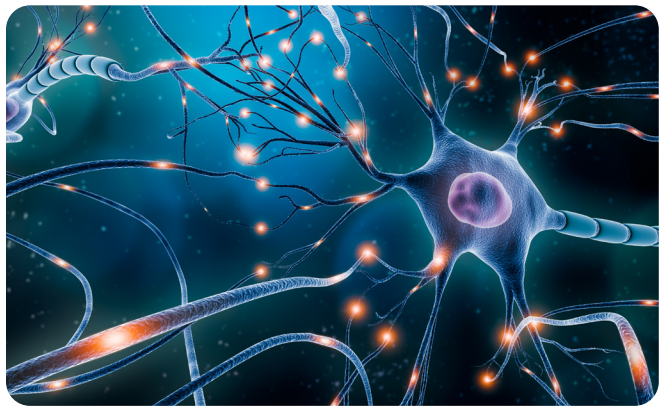
Tracking neuroinflammation using transgenic mouse models and optical imaging

Introduction

Amyotrophic lateral sclerosis (ALS) is a devastating neurological disease for which there is no cure. It involves the progressive degeneration of motor neurons leading to muscle weakness, cramps, twitching, and eventually paralysis of the limbs and trunk. Death usually results 1-5 years following diagnosis.[1] Another lethal brain disorder is stroke, which occurs when blood supply to the brain is disrupted by a blood vessel that bursts or becomes blocked.

Neuroinflammation plays a key role in both of these diseases as well as in the pathogenesis for Alzheimer's, multiple sclerosis, and other forms of brain injury.[2] The ability to monitor activation of microglia, the innate immune cells of the central nervous system, is of paramount importance to understanding neuroinflammatory disease development, progression, and response to therapy.

Dr. Jasna Kriz, Professor in the Department of Psychiatry and Neurosciences at Laval University in Quebec, has spent much of her career finding better ways to study the initiation and progression of diseases like ALS and stroke. The main strategy of her lab has been to develop transgenic mouse models for optical imaging of various cells in the brain: astroglia, microglia, and neurons.[3] The mice have been modified to express firefly luciferase when immune processes are activated in the brain and enable study of important brain diseases via 2D and 3D bioluminescence imaging.



The biological challenge

Monitoring functional changes in a disease model over time proves challenging. Conventional imaging modalities render excellent structural information but cannot always deliver information about biological changes at the molecular level. PET scans provide molecular information but are costly and not feasible for some labs to conduct for their exploratory preclinical research. Bioluminescence imaging, on the other hand, is more economical and provides real-time information regarding expression of genetic reporters as well as changes in disease markers over time. The challenge that Dr. Kriz undertook was figuring out how to develop mouse models that would luminesce at sites of neuroinflammation.

Dr. Kriz and her team targeted several markers for neuroinflammatory status, including toll-like receptor 2 (TLR2) and glial fibrillary acidic protein (GFAP). TLR2 is a pattern recognition receptor for ligands called "alarmins" that indicate tissue damage, infection, and possible tumor cells.[4] TLR2 is expressed on microglia and its activation is a key mediator of neuroinflammation.[5] GFAP has been found to be a hallmark of neurodegenerative diseases such as Parkinson's, Alzheimer's,

revvity

ALS, and stroke.[6] It's expressed almost exclusively in astrocytes making it an ideal biomarker to study inflammation specific to the CNS. GFAP expression increases rapidly during a process called "reactive astrogliosis" which occurs in response to acute injury and other perturbations in the brain. GFAP can also be found in non-myelinating Schwann cells that perform functions similar to astrocytes.

There were many key questions Dr. Kriz and her colleagues hoped to address including: How does neuroinflammation change in various diseases over time? How do neuroinflammatory diseases respond to inhibitor therapies? Are changes in neuroinflammation correlated with neurological function? Can we visualize silent, presymptomatic disease? The hope was that by linking bioluminescent reporters with neuroinflammatory markers, these questions could be addressed non-invasively over long observational periods.

How optical imaging was used

Dr. Kriz and her colleagues have developed transgenic mouse models to optically follow specific cell types involved in inflammation and neuronal changes in models of neurodegenerative disease and brain injury. The models include: GFAP-Luc (for astroglia & Schwann cells), TLR2-Luc/GFP (for microglia), and Gap43-Luc/GFP (for neurons). Dr. Kriz and her team have become proficient in creating dual-tagged models, namely with luciferase and green fluorescent protein (GFP) to facilitate *in vivo* and *ex vivo* imaging, respectively.

Our Challenge

"There are many advantages to *in vivo* optical imaging. It is a form of live animal pathology that can inform on disease status longitudinally in therapeutic studies. It also acts as a bridge between basic and translational research.

Our greatest challenge was being able to evaluate very early neuroinflammatory disease events. The IVIS® Spectrum imaging system combined with bioluminescent reporters provided such high sensitivity, we were able to detect biological changes prior to the onset of symptoms."

Dr. Jasna Kriz

Neuroinflammation is induced either by genetic modification or injection of toxins. The transgenic mice are predisposed to developing neurodegenerative diseases so that neuroinflammation can be studied both before and after symptoms manifest. Alternatively, models of neuroinflammation were also achieved by chemical induction using lipopolysaccharide (LPS) which is a bacterial endotoxin commonly used to induce inflammation in both the central and peripheral nervous systems. Acute inflammation is achieved with a single intraperitoneal injection of LPS. Chronic inflammation is achieved by serial injections over time.

In stroke models, they discovered that microglial activation after stroke is a chronic response and persists for several months after ischemic injury. The study of GFAP-Luc transgenic mice also revealed sex-specific injury markers after ischemic insult that are part of early inflammatory responses (Fig 1).[7] In addition, they noted inflammatory activation in other parts of the brain that were not directly affected by the stroke indicating that the effect of an ischemic event may be more widespread than originally thought. They investigated additional brain biomarkers, including nestin, a known marker of multipotent stem cells. Literature precedents suggested that the nestin-positive progenitor cells may differentiate into GFAP-positive astrocytes and Dr. Kriz and her colleagues were able to corroborate these results in models of both acute and chronic neuroinflammation.[8] As mentioned previously, TLRs are another important receptor family that are expressed on microglia and mediate neuroinflammation after stroke-induced necrosis. Transgenic mice with TLR2 loss of function were developed to understand both beneficial and harmful effects of having modulated neuroinflammation. In Fig 2, LPS was used to initiate inflammation in the brain and spinal cord of TLR2-Luc transgenic mice which was examined using 3D bioluminescence imaging on the IVIS® Spectrum system.

The power of 3D imaging

"The 3D imaging capabilities of the IVIS Spectrum optical imaging system have allowed us to pinpoint exactly when and where neuroinflammatory events arise in the CNS. When combined with our transgenic reporter mice, optical imaging has been a powerful tool to help us answer questions related to longitudinal changes in neuroinflammation for a range of brain diseases."

In ALS models, bioluminescence imaging using Revvity's IVIS Spectrum system was used to study patterns of disease spreading. ALS is known to start focally and then spread in ways that are not well understood. They used a GFAP-luc/SOD1G93A reporter mouse to investigate the sequence of pathogenesis in the spinal cord and periphery and which cell types are first to be affected.[9] Imaging revealed periods of upregulated GFAP promoter activity in the spinal cord. The first wave of upregulation occurred at only 5 weeks of age - before the onset of symptoms. Two additional spikes in GFAP promoter activity occurred at 16 weeks (mice experience hind limb paralysis) and 20 weeks (end stage of disease). GFAP induction was also noted in peripheral nerve Schwann cells and correlated closely with disease onset. Using their transgenic models, they have also discovered that the cerebrospinal fluid (CSF) plays a greater role than previously expected in the spread of ALS pathogenesis as well as evaluated the efficacy of new inhibitor therapies.[10-12]

In an Alzheimer's model, GFAP expression was monitored using bioluminescence (Fig 3). The extent of neuroinflammation was measured over the course of 15 months and revealed marked enhancement in the Alzheimer's cohort compared to the wild type control with the discrepancy growing over time.

Optical imaging was also used to monitor microglial response to electrical brain stimulation which is currently being tested as a treatment for neuropathic pain, a symptom of both stroke and Alzheimer's.[13] TLR2-fluc-GFP transgenic mice, revealed localized TLR2 signal in the 3 days following surgery which then decreased and remained stable after day 6. They proceeded with electrical stimulation two weeks after electrode implantation using a biconcentric electrode to ensure local stimulation. TLR2 signal increased by roughly 5-fold over baseline on day 3 and returned to near-baseline level by day 9. The IVIS Spectrum was used to obtain 3D bioluminescence images that were used to confirm that the optical source was deep within cortical layers and were not resulting from the superficial tissue where surgery was conducted.

The use of 3D imaging in the ALS and Alzheimer's models was imperative to determine the location of GFAP expression. 2D surface radiance is impacted by scattering, diffusion, and attenuation of photons as they travel through tissue, thus exact determinations about *in vivo* source location purely based on 2D data is very risky to assume in deeper tissue areas. The 3D algorithms consider what happens to light at depth in tissue, and the resulting data can be more accurate in terms of localization, quantification, and colocalization.

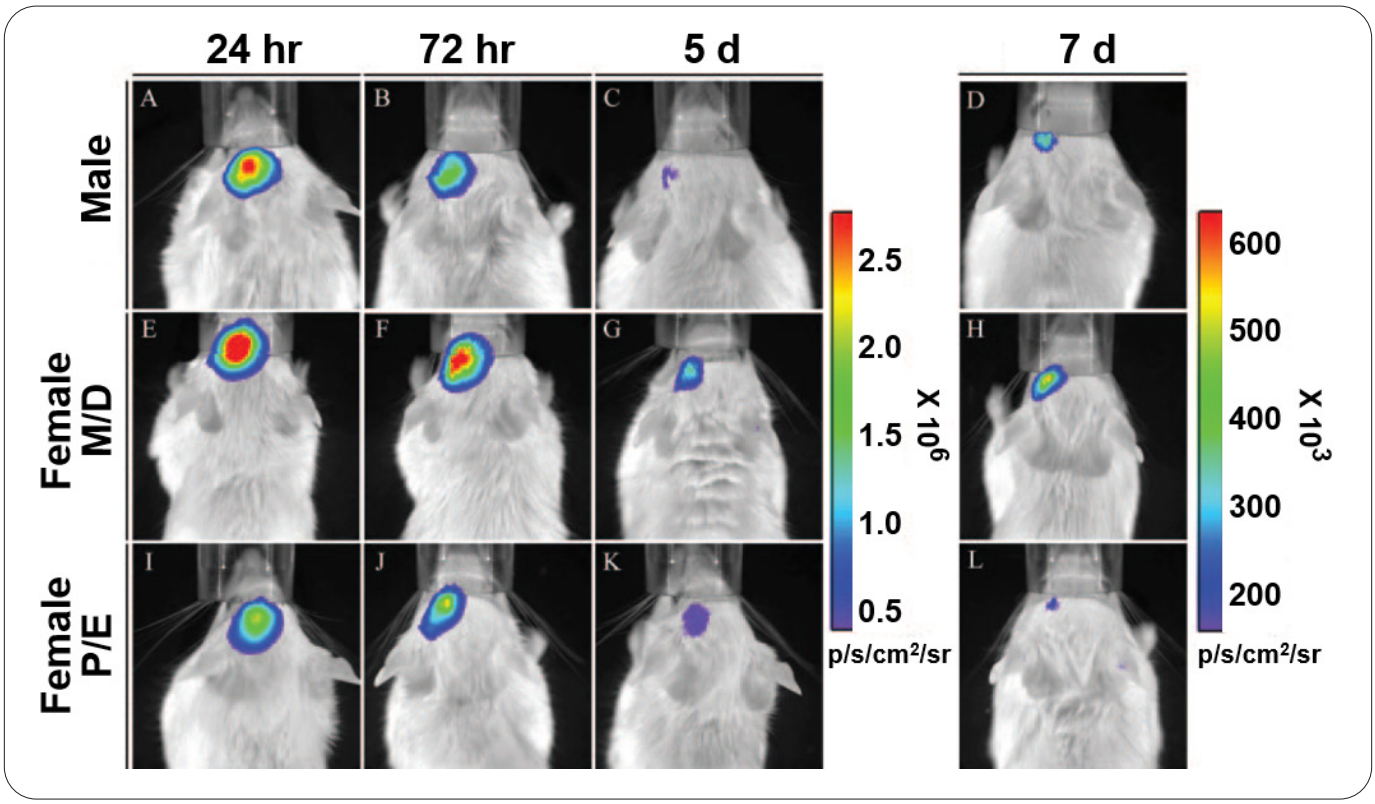


Figure 1. 2D Bioluminescence imaging in a mouse stroke model. GFAP-Luc transgenic mice show sex differences in mice during the initial recovery after ischemic injury. Imaging reveals an estrogen-dependent modulation of GFAP signals in female mice. M/D = metestrus/diestrus. P/E = proestrus/estrus. Imaged using the IVIS system.

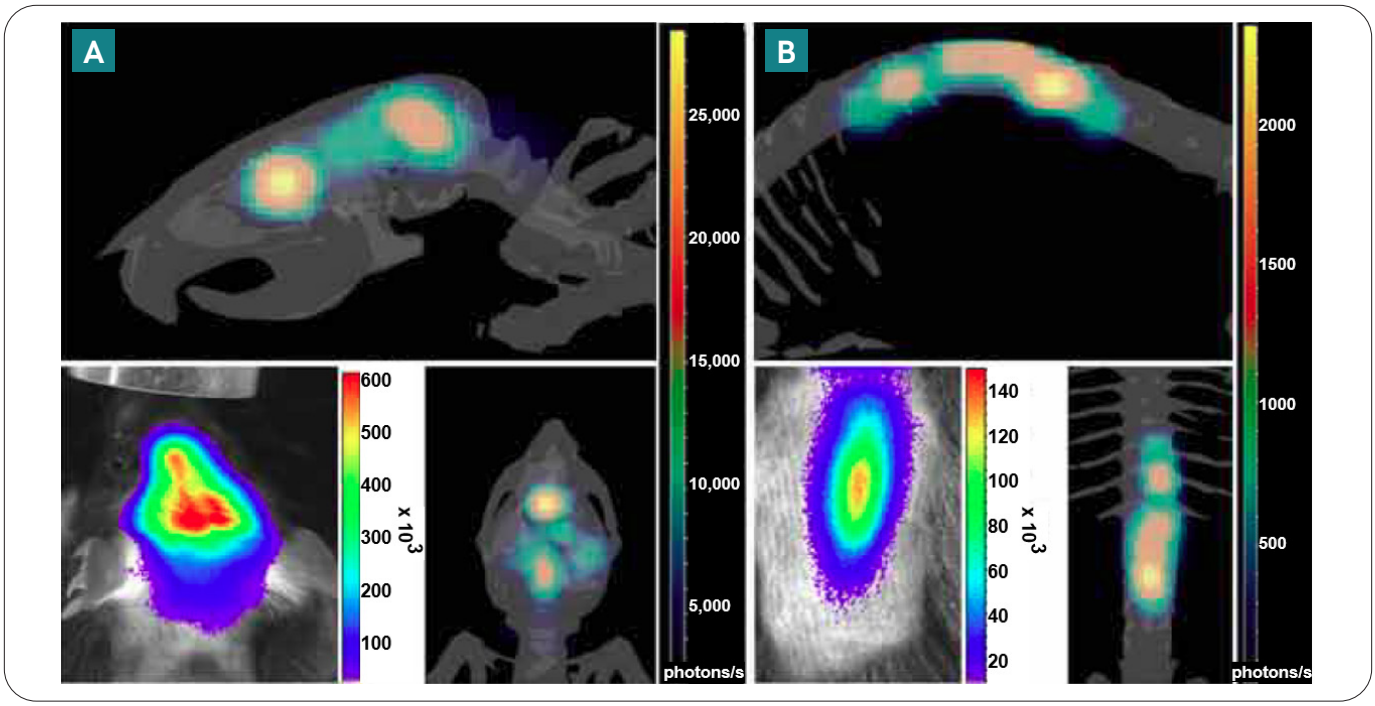


Figure 2. 3D Bioluminescence imaging of TLR2-Luc mouse. The TLR2-Luc transgenic mouse model was systemically challenged with lipopolysaccharide (LPS) to induce inflammatory response. After 24 hours, 3D BLI was performed to identify areas of CNS inflammation within the (A) brain and (B) spinal cord. Imaged using the IVIS Spectrum.

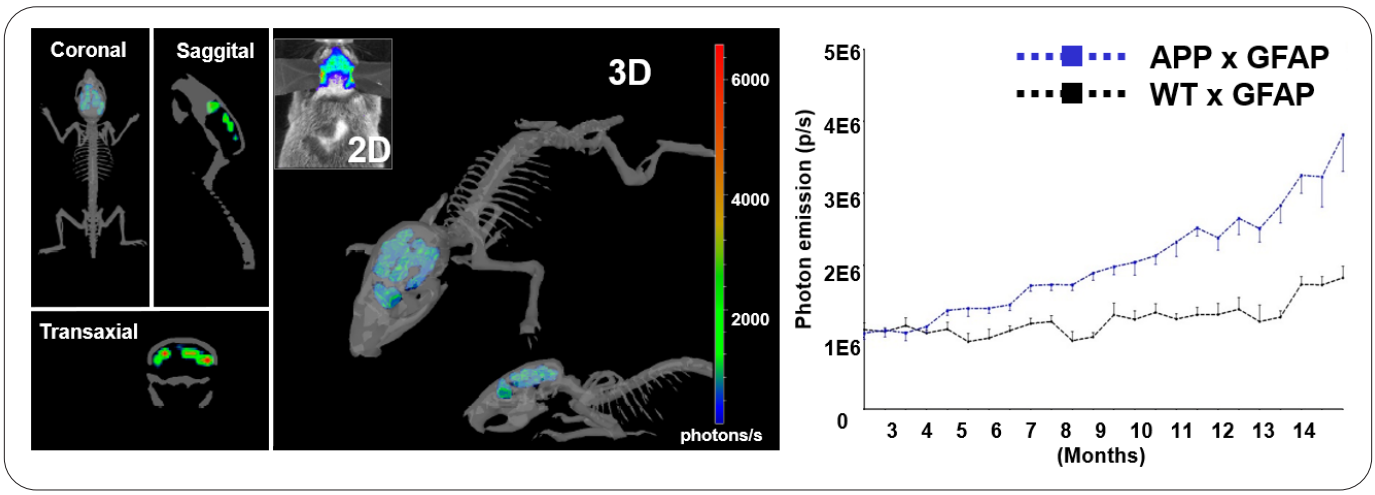


Figure 3. 3D Bioluminescence imaging and quantification in an Alzheimer's disease mouse model. Transgenic mice were developed with bioluminescence tag for glial fibrillary acidic protein (GFAP) to monitor neuroinflammation/astrocyte activation. Wild type (WT) mice were compared to mice presenting with amyloid precursor protein (APP), a marker for Alzheimer's. Imaged using the IVIS Spectrum system.

Conclusion

Using novel luciferase-expressing transgenic mouse models, Dr. Kriz and team have utilized the sensitivity of the IVIS Spectrum optical imaging system to uncover biological mechanisms underpinning the development and progression of neurodegenerative diseases and brain injury. They have investigated the spread of disease from very early stages, mapping out processes that occur before the onset of symptoms, and identified specific cell types that contribute to disease progression. In addition, they used bioluminescence imaging to evaluate therapeutic efficacy of molecular inhibitors for neuroinflammatory pathways and to evaluate biological responses such as microglial activation to electrical brain stimulation. Taken together, these models are powerful tools for longitudinal study of the biological consequences of acute brain injuries as well as chronic neurodegenerative diseases.

IVIS 2D & 3D pptical imaging platform offers:

- High sensitivity 2D and 3D *in vivo* bioluminescence and fluorescence imaging
- High throughput and high-resolution (20 microns in 3.9 cm field of view) options for *in vivo*, *ex vivo*, and *in vitro* applications
- 3D diffuse tomographic reconstruction allowing for pinpoint localization of optical signal
- Ability to automatically co-register CT or MRI images to provide anatomical context for your optical data

References

1. Cleveland, D. W.; Rothstein, J. D. From Charcot to Lou Gehrig: Deciphering Selective Motor Neuron Death in ALS. Nat. Rev. Neurosci. 2001, 2 (11), 806–819. https://doi.org/10.1038/35097565.
2. DiSabato, D. J.; Quan, N.; Godbout, J. P. Neuroinflammation: The Devil Is in the Details. J. Neurochem. 2016, 139 Suppl 2, 136–153. https://doi.org/10.1111/jnc.13607.
3. Cordeau, P.; Kriz, J. Real-Time Imaging after Cerebral Ischemia: Model Systems for Visualization of Inflammation and Neuronal Repair. Methods Enzymol. 2012, 506, 117–133. https://doi.org/10.1016/B978-0-12-391856-7.00031-7.
4. De Oliviera Nascimento, L.; Massari, P.; Wetzler, L. M. The Role of TLR2 in Infection and Immunity. Front. Immunol. 2012, 3. https://doi.org/10.3389/fimmu.2012.00079.
5. Hoffmann, O.; Braun, J. S.; Becker, D.; Halle, A.; Freyer, D.; Dagand, E.; Lehnardt, S.; Weber, J. R. TLR2 Mediates Neuroinflammation and Neuronal Damage. J. Immunol. 2007, 178 (10), 6476–6481. https://doi.org/10.4049/jimmunol.178.10.6476.
6. Yang, Z.; Wang, K. K. W. Glial Fibrillary Acidic Protein: From Intermediate Filament Assembly and Gliosis to Neurobiomarker. Trends Neurosci. 2015, 38 (6), 364–374. https://doi.org/10.1016/j.tins.2015.04.003.
7. Cordeau, P.; Lalancette-Hébert, M.; Weng, Y. C.; Kriz, J. Live Imaging of Neuroinflammation Reveals Sex and Estrogen Effects on Astrocyte Response to Ischemic Injury. Stroke 2008, 39 (3), 935–942. https://doi.org/10.1161/STROKEAHA.107.501460.
8. Krishnasamy, S.; Weng, Y.-C.; Thammisetty, S. S.; Phaneuf, D.; Lalancette-Hebert, M.; Kriz, J. Molecular Imaging of Nestin in Neuroinflammatory Conditions Reveals Marked Signal Induction in Activated Microglia. J. Neuroinflammation 2017, 14 (1), 45. https://doi.org/10.1186/s12974-017-0816-7.
9. Keller, A. F.; Gravel, M.; Kriz, J. Live Imaging of Amyotrophic Lateral Sclerosis Pathogenesis: Disease Onset Is Characterized by Marked Induction of GFAP in Schwann Cells. Glia 2009, 57 (10), 1130–1142. https://doi.org/10.1002/glia.20836.
10. Mishra, P. S.; Boutej, H.; Soucy, G.; Bareil, C.; Kumar, S.; Picher-Martel, V.; Dupré, N.; Kriz, J.; Julien, J.-P. Transmission of ALS Pathogenesis by the Cerebrospinal Fluid. Acta Neuropathol. Commun. 2020, 8 (1), 65. https://doi.org/10.1186/s40478-020-00943-4.
11. Kumar, S.; Phaneuf, D.; Cordeau, P.; Boutej, H.; Kriz, J.; Julien, J.-P. Induction of Autophagy Mitigates TDP-43 Pathology and Translational Repression of Neurofilament MRNAs in Mouse Models of ALS/FTD. Mol. Neurodegener. 2021, 16 (1), 1. https://doi.org/10.1186/s13024-020-00420-5.
12. Gravel, M.; Béland, L.-C.; Soucy, G.; Abdelhamid, E.; Rahimian, R.; Gravel, C.; Kriz, J. IL-10 Controls Early Microglial Phenotypes and Disease Onset in ALS Caused by Misfolded Superoxide Dismutase 1. J. Neurosci. Off. J. Soc. Neurosci. 2016, 36 (3), 1031–1048. https://doi.org/10.1523/JNEUROSCI.0854-15.2016.
13. Arsenault, D.; Drouin-Ouellet, J.; Saint-Pierre, M.; Petrou, P.; Dubois, M.; Kriz, J.; Barker, R. A.; Cicchetti, A.; Cicchetti, F. A Novel Combinational Approach of Microstimulation and Bioluminescence Imaging to Study the Mechanisms of Action of Cerebral Electrical Stimulation in Mice. J. Physiol. 2015, 593 (Pt 10), 2257–2278. https://doi.org/10.1113/jphysiol.2014.287243.

Revvity, Inc
940 Winter Street
Waltham, MA 02451 USA

(800) 762-4000
www.revvity.com