ORGANOTYPIC BRAIN SLICE CULTURES AS TOOLS TO MODEL NEUROLOGICAL DISEASES AND FOR FUNCTIONAL SCREENING OF DRUGS

Roberta De Simone (a), Caterina Veroni (b), Luisa Minghetti (c), Maria Vinci (d),
Maria Antonietta Ajmone-Cat (a)
(a) National Centre for Drug Research and Evaluation, Istituto Superiore di Sanità, Rome, Italy
(b) Department of Neuroscience, Istituto Superiore di Sanità, Rome, Italy
(c) Research Coordination and Support Service, Istituto Superiore di Sanità, Rome, Italy
(d) Department of Onco-Hematology, Gene and Cell Therapy, Bambino Gesù Children's Hospital, Rome, Italy

Introduction

The 3Rs approach (Replacement, Reduction, and Refinement) is at the basis of national and international legislation and regulations to improve welfare of animals used in research. Although *in vivo* animal models represent the most realistic platform for research and testing of therapeutic approaches, they may also imply stress and/or pain for the animals. This raises ethical issues and arouses intense debate on their necessity, justification, and acceptability. Moreover, *in vivo* studies require considerable financial and time efforts to set up, to be performed and analysed.

On the other hand, *in vitro* cell-based studies fail to replicate the conditions of cells in a tissue, sometimes weakening the predictive value of *in vitro* data. Recent advances in three-dimensional (3D) culture techniques have provided a bridge to fill the gap between *in vivo* procedures and *in vitro* methods, enabling the study of tissue and organ function *ex vivo*. Many successful examples of reconstituting organ and tissue function *ex vivo* are now available for most tissues and organs (Shamir & Ewald 2014), including brain.

Organotypic Brain Slice Cultures (OBSCs) substantially retain the morphological and physiological features of the brain region of origin, including cell-cell interactions, neuronal networks, and synaptic organization, and contain nearly all brain cell types, thus allowing to study different cell populations within their microenvironment.

These cultures can be successfully prepared from almost any region of the Central Nervous System (CNS); however, hippocampus, cerebellum and spinal cord have been so far the most widely studied for their clear implication in several neurological and neuropsychiatric disorders. Co-cultures of two or more regions that take part in a pathway, as well as whole brain coronal or sagittal slices, can also be established to mimic a more *in vivo*-like environment, although their long-term culturing is more challenging and requires specific medium adaptations.

OBSCs are typically prepared from early postnatal rodents (usually P5-8), as brain slices from embryonic and adult stages are more vulnerable to mechanical trauma during preparation and easily lose their cytoarchitectural organization in the long-term. These cultures are suitable for any kind of application and analytical technique (including molecular and biochemical assays, long-term live imaging, or electrophysiology), making them an excellent model system for several areas of neuroscience field.

OBSCs represent a valid method of partial Replacement, which includes "the use of primary cells (and tissues) taken from animals killed solely for this purpose (i.e., not having been used in a scientific procedure that causes suffering)", according to the definition formulated by the UK's National Centre for the Replacement, Refinement and Reduction of animals in research (NC3R).

OBSCs have also been prepared from *Drosophila*, in further fulfilment of partial Replacement (which also includes "the use of some animals that, based on current scientific thinking, are not considered capable of experiencing suffering"), and from human patients, in the fulfilment of the full Replacement principle. However, translational limitations of the circuitry modelled in the fruit fly ganglia in the first case, and technical and methodological limitations in the second, including poor and sporadic availability of human tissues, currently hinder their widespread use.

An additional benefit of OBSCs is related to Reduction, as multiple slices can be prepared from one single animal and used in different experiments, significantly reducing the number of animals to be euthanized compared to an *in vivo* experiment.

Moreover, experimental procedures causing distress and/or pain to the animal, including traumatic lesions, administration of drugs, exposure to neurotoxicants, induction of inflammation, neurodegeneration/neuropathology, demyelination, can be directly applied to the slices, in the fulfilment of the Refinement principle. The recent possibility of genetically manipulating slices after explant, by several transfection techniques, further increases the perspective for expanding the use of these models in neuroscience studies, according to the Refinement principle. For example, the use of recombinant adeno-associated virus (rAAV)-mediated gene delivery to selectively manipulate neurons, astrocytes, microglia, oligodendrocytes, or combinations thereof, now allows to explore cell-autonomous and non-cell-autonomous mechanisms in the different experimental conditions modelled by OBSCs.

Conversely, target innervation and the cross-talk with the systemic milieu and peripheral organs (e.g., pituitary and adrenal glands, intestine) are not represented in OBSCs; similarly, the absence of functional vasculature and intact blood-brain-barrier must be considered when considering drug candidates through to further pre-clinical testing. Future efforts in combining OBSC models with microfluidic engineering technology (such as in body-on-a chip systems) could be the key to increase the translational value of this powerful *ex vivo* platform. Other limitations of the OBSC model include the acute effects of axotomy and slice flattening over time, which can be at least partially overcome by optimization of temperature and incubation media. At the same time, post-dissection events per se can be studied as a model of brain injury and recovery.

Moreover, the initial inflammatory reaction spontaneously subsides after 10-14 days from explant, and the slices are ready to be exposed to different experimental protocols and analysed by biochemical, morphological, and functional assays after one or two additional weeks.

Here we provide some examples of the exploitation of rodent OBSC models, currently used in our laboratories, to investigate the complexity of the healthy and pathological brain.

Organotypic hippocampal slice cultures as a model to study microglial functional properties and neuroinflammatory processes

Accumulating evidence is showing that neuroinflammation, mainly sustained by the resident immune cells microglia, is involved in a broad context of acute and chronic neurological diseases, including stroke, brain's response to traumatic injury, Alzheimer's disease, Parkinson's disease, epilepsy, Multiple Sclerosis (MS). Moreover, neuroinflammation is a key player in the development of cognitive and emotional dysfunctions in several psychiatric disorders, including anxiety and depression (Gilhus & Deuschl, 2019).

Besides its critical role in host defence and pathology, the brain immune system modulates homeostatic brain processes such as synaptic formation and scaling, long-term potentiation, and neurogenesis. Thus, aberrant microglial activation during critical windows of development may result in neurodevelopmental defects and increased vulnerability to inflammatory stimuli, setting the stage for adult psycho- and neuro- pathologies.

It is now largely accepted that microglia in response to different cues can acquire an array of different functional states, well beyond the classical pro-inflammatory and the alternative anti-inflammatory states, traditionally referred to as M1 and M2 phenotypes, respectively.

The acquisition of the different phenotypes is a dynamic process, whose modulation is likely related to diverse outcomes of microglial activation in terms of brain repair or damage. Another important concept is the existence of "innate immune memory" in microglia, whereby phenomena such as "priming" and "tolerance/sensitization" occur (*see* Neher & Cuningham, 2019 for a recent review).

"Priming" takes place when the exposure to an initial stimulus makes microglia more susceptible to a delayed secondary inflammatory stimulus, which can then trigger a heightened or exaggerated inflammatory response.

"Tolerance/sensitization" is characterized by suppression of pro-inflammatory gene expression on one hand and induction of anti-inflammatory genes on the other, in response to persistent stimulation.

There is still scarce information available on the trajectory of microglial activation in different pathological conditions, on the reversibility of different phenotypic commitments, on the ability to preserve the molecular memory of previous stimuli, and therefore on the functional consequences on the viability and fate of other brain cell populations.

OBSs provide an ideal tool to study these aspects, as they enable the analysis of microglial responses against any treatment in an *in vivo*-like microenvironment and, in parallel, of relative effects of each treatment on the properties of mature and immature neuronal and glial cells (astrocytes and oligodendrocytes), over extended time in culture.

As an example of such an application, we used Organotypic Hippocampal Slice Cultures (OHSCs) (Figure 1) from rat pups (P5-P6), prepared according to the membrane interface technique (350-400 μm thick), to get insight into the process of microglial activation in response to single vs repeated exposure to the bacterial endotoxin lipopolysaccharide (LPS).

LPS, along with several other components of pathogens and endogenous molecules produced in conditions of tissue damage, is specifically recognized by Toll like receptor 4 (TLR4), a key component of the innate immune system belonging to the Pattern Recognition Receptor (PRR) family; LPS is thus a prototypical inflammogen, widely used in studies on central and peripheral inflammation.

Differently from a single LPS challenge, persistent LPS stimulation of OHSCs elicited a potentially protective microglial response, characterized by the elevated expression of anti-inflammatory and neuroprotective molecules (Ajmone-Cat *et al.*, 2013), thus validating and extending, in a more complex physiological context, the data previously obtained in purified microglial cultures (Ajmone-Cat *et al.*, 2003; Cacci *et al.*, 2008). Thanks to their long-lasting viability (up to 5-6 weeks) and ease accessibility, OHSCs offered the possibility to study the consequences of these "acute" or "chronic" preconditioning paradigms on the response to a new hit. This approach evidenced the existence of mechanisms of molecular inflammatory memory that can be relevant in pathological brain conditions characterized by chronic or repeated exposure to pathological noxiae. Important microglial functional aspects, such as phagocytic activity (Figure 1c-d) and motility, could also be analysed into OHSCs (Ajmone-Cat *et al.*, 2013).

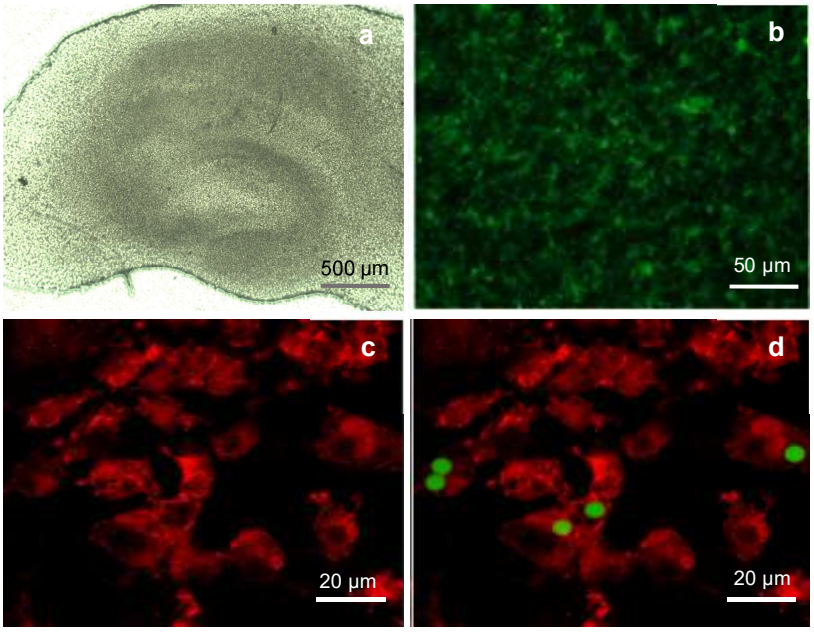

Figure 1. Microglial reactivity in rat organotypic hippocampal slice cultures (OHSCs): a) Phase contrast microphotograph of organotypic hippocampal slice cultures (OHSCs) from rat pups (P5-P6), imaged at 2 weeks post-dissection. Scale bar = 500 µm. b) Fluorescent staining of microglia in unstimulated OHSC, labelled for the microglial marker Iba-1 (green). Scale bar = 50 µm. c-d) Confocal images showing the ingestion of fluorescent latex beads (6 µm-diameter, green) by Iba-1 (red) positive microglia in OHSCs (modified from Ajmone-Cat *et al.* 2013)

By taking advantage of the same experimental model, we identified glycogen synthase kinase-3 beta (GSK-3β), a crucial regulator of TLR signalling, as a part of the molecular machinery involved in the adaptive response of microglia to repeated LPS stimulations. Inhibition of GSK-3β by the mood-stabilizer drug lithium chloride (LiCl) enhanced the process of microglial adaptation to repeated stimuli, favouring the acquisition of anti-inflammatory/protective functions and the extinguishing of pro-inflammatory ones. Oligodendrocyte maturation was higher in slices exposed to repeated LPS stimulation than in slice exposed to a single LPS or naive, and further enhanced in the presence of LiCl, as indicated by the expression of Gpr17 and Myelin Basic Protein (MBP), markers of oligodendrocyte maturation (Ajmone-Cat *et al.*, 2016). This and other evidence reinforced the – at that time – emerging idea that the outcome of microglial activation can be protective and favourable to regenerative/reparative mechanisms under specific conditions, and not univocally harmful, as it was previously conceived.

Collectively, this and other studies prove that OHSCs constitute a powerful *ex vivo* tool for dissecting diverse aspects of neuroinflammation at various cellular and molecular levels, and for testing compounds able to modulate microglial reactivity towards protective functions. OHSs, and OBSs in general, are attracting more and more attention due to the possibility of applying and combining new experimental approaches. For example, the application of genetic and/or pharmacological techniques to deplete microglia by OHSCs, followed by microglia replenishment with adoptive transfers, is providing additional insight into microglial biology (Coleman *et al.*, 2020).

More recently, OBSs (hippocampal and hippocampal-entorhinal cortex slices) from adult mice, including genetically engineered mice, have been employed to study aspects of aging-

related neuropsychiatric/neurodegenerative disorders (Humpel *et al.*, 2019), as well as for high throughput screening of potential agents working against such disorders. In addition, behavioural data from specific mouse strains can be correlated with data obtained from the slices of the same animals, such as molecular, functional, or morphological data, increasing the translational relevance of this experimental platform.

Whole brain organotypic brain slice culture as a model to study tumor biology

Pediatric Glioblastoma (pGBM) and Diffuse Intrinsic Pontine Glioma (DIPG) are amongst the most aggressive tumors of the CNS, characterized by a significant degree of genetic and phenotypic intra-tumor heterogeneity, affecting children and young adults, for which there is no effective treatment (Vinci *et al.*, 2018).

Understanding the mechanisms that regulate the direct or indirect cell-cell communication within genetically and phenotypically distinct subclonal cell populations and with the brain microenvironment could allow the identification of effective therapeutic strategies against these fatal diseases.

We have recently developed a new model of whole brain organotypic slice cultures (wOBS; sagittal or coronal), based on an adaptation of the previously published hippocampal brain slice approach (Ajmone-Cat *et al.*, 2013), to establish a co-culture system consisting of wOBSs with pGBM or DIPG primary patient-derived cell lines (Figure 2).

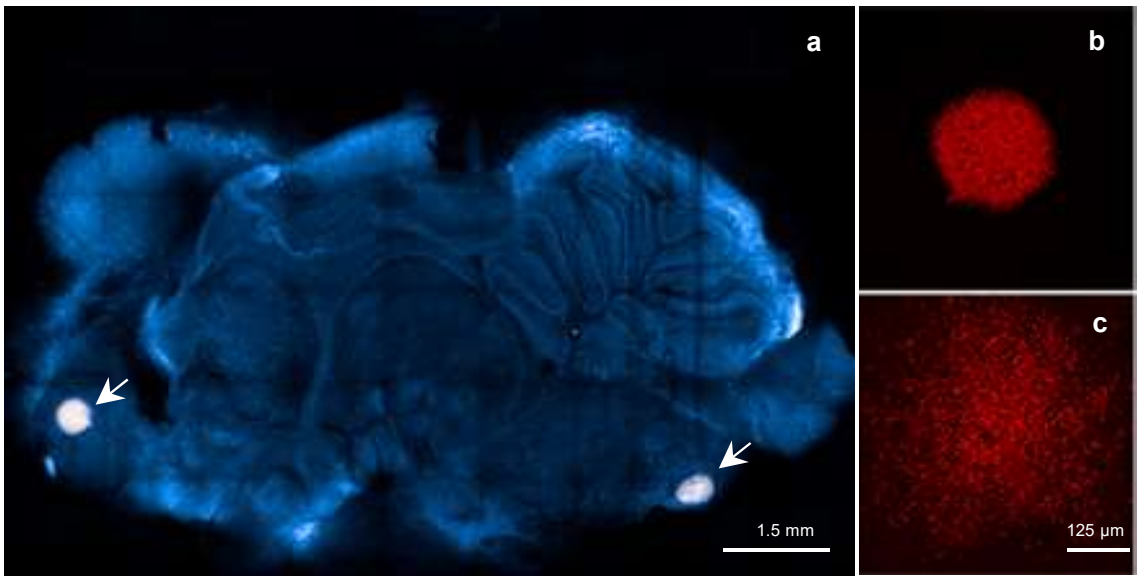

Figure 2. Co-culture of whole brain organotypic slices with primary patient-derived glioma cells: a) In whole brain sagittal organotypic slice (wOBS; 350 µm thick) from CD1 mouse pup (PND 6–7), neurospheres obtained from a DIPG primary-patient derived cell line were implanted into the cortex and pons (arrows). The image was obtained by Operetta CLS (mosaic 10X). Up to six slices, complete of pons and medulla, can be obtained from each brain and cultivated for at least 3 weeks. b-c) Confocal picture of implanted cells at day 1 (b) and day 7 (c) post-implantation, stained with anti-human nuclei antibody

Subsequently, multifluorescent marking technology was applied by Vinci's group to derive single cell-derived clones and study their heterogeneous invasion into wOBS once implanted as neurospheres in the pontine area. Different fluorescence analysis platforms (e.g., Confocal microscope, Operetta CLS and Digital slide scanner) were integrated to image and analyse the obtained DIPG/wOBS cultures (Pericoli *et al.*, 2020).

This model will enable future investigations into the cellular and molecular mechanisms of intra-tumor heterogeneity in pGBM and DIPG and will allow exploring how different clones can interact with the microenvironment, including tumor associated-microglia. Tests of promising anti-tumoral compounds, or their combination with emerging approaches such as CAR-T cells therapy, are underway in our laboratories using this model (de Billy *et al.*, 2021), opening new avenues to accelerate translational research in this field.

Organotypic cerebellar slices as a model for the study of myelination/remyelination processes

Myelin sheath consists of lipid-rich membrane layers wrapped around axons, allowing rapid propagation of action potentials, providing metabolic support to neurons and maintaining axonal integrity. Axon myelination occurs after the differentiation of Oligodendrocyte Progenitors (OPs) in mature oligodendrocytes, the myelin-producing cells of the CNS, and implicates a multistep process finely tuned by intrinsic or environmental factors. Demyelinating disorders, characterized by a chronic or episodic destruction of the myelin sheath resulting in impaired neuronal function, are a leading cause of neurological disability with a major impact on quality of life and public health costs. In MS, a chronic inflammatory disease of the CNS, myelin damage is followed by spontaneous remyelination, however as the disease progresses, this recovery process ultimately fails and persistent demyelination with consequent axonal loss results in progressive and irreversible functional deficits (Franklin *et al.*, 2020). Currently approved therapeutic strategies for MS include a variety of anti-inflammatory and immunomodulatory treatments that effectively control the rate of relapse and delay disease progression; however, therapies that can promote remyelination are not yet available and the molecular and cellular basis of the myelin repair deficiency during the progression of MS are still unclear (Cunniffe & Coles, 2021). Understanding how oligodendrocytes initially produce myelin during development and remyelinate axons in the diseased CNS is of significant clinical interest, as it paves the way for novel possible strategies to stimulate endogenous OP differentiation as means of myelin repair and neuroprotection (Franklin *et al.*, 2020). In recent years, the molecular mechanisms regulating OP activation and differentiation into mature myelinating oligodendrocytes have been extensively studied using both *in vitro* and *in vivo* models, and a reasonable number of interesting drug targets and putative chemical modulators impacting remyelination have been identified.

Also, in this field of research, *ex vivo* cultures represent an excellent compromise between the advantages and disadvantages of both *in vitro* and *in vivo* systems described in the introduction. Cerebellar slice cultures from neonatal laboratory rodents have indeed emerged as a very useful and accessible model to study the molecular and cellular mechanisms regulating OP biology, as well as myelination, demyelination, and remyelination of axons, while maintaining cross-talk between neural cell populations in the context of a preserved cytoarchitecture (Doussau *et al.*, 2017).

It has been shown that slices prepared from post-natal day 7 rodent brains preserve a relative intact three-dimensional cellular environment and all stages of the oligodendrocyte lineage are maintained during subsequent development *in vitro*. In addition, MBP transcript increases over

time (Figure 3A, unpublished data) and partial myelination can be observed after 5 days *in vitro* as revealed by the significant number of MBP-positive processes aligned with axons (Figure 3B, unpublished data).

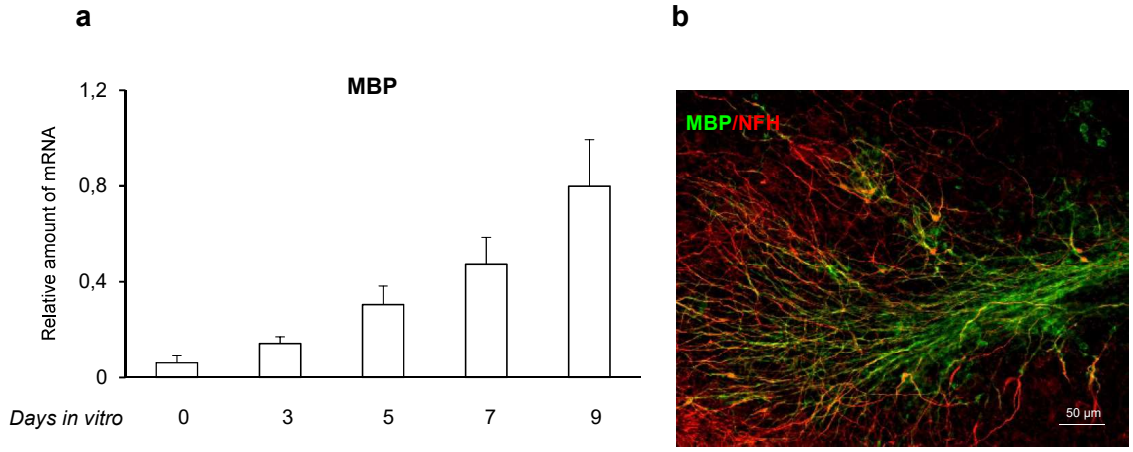

Figure 3. Characterization of cerebellar slice myelination model: a) Expression of myelin basic protein (MBP) transcript in cerebellar slices during myelination: slices were prepared by 7-day-old mouse cerebellum and grown in culture medium. MBP transcripts increased linearly during the developmental period analysed. Data are expressed as induction of MBP mRNA normalized to GAPDH (2^-ΔCt) + standard error of the mean of 3 experiments. b) Immunohistochemistry characterization of cerebellar slice myelination model: myelin basic protein (MBP, green) and neurofilament heavy chain (NFH, red) staining in organotypic cerebellar slices prepared from P7 mouse cerebellum after 5 days in culture shows preserved neurons and myelin processes aligning with axons

Based on this evidence, myelinating slices represent a powerful tool to study oligodendrocyte maturation and differentiation over time as well as their response to different stimuli influencing the first stages of myelin development. Demyelination of axons in cerebellar organotypic slices can be induced using the membrane-disrupting chemical lysolecithin, resulting in a rapid loss of myelin, with the subsequent return of myelin sheaths after toxin removal, suggestive of remyelination. This model has been extensively characterized both at protein and gene expression level demonstrating that after toxin removal and during the recovery phase OPs proliferate and differentiate in myelin-producing oligodendrocytes and macrophages are present and activated to clear myelin debris (Zhang *et al.*, 2011; Veroni *et al.*, 2020). Both myelination and demyelination/remyelination models can answer a number of questions. Indeed, they can be used for different experimental approaches, which include the study of factors that are regulated during the processes of myelin development, damage and repair, the analysis of the electrical activity and potential changes of neurons, the possibility to apply genetic manipulation by preparing slices from transgenic mice or using viral vectors and other innovative tools such as CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) and small RNA interference. Recently, a model in which exogenous OPs are cultured in organotypic mouse cerebellar slices depleted of their endogenous oligodendrocytes has been described (Baudouin *et al.*, 2021), offering the challenging perspective of analysing human OP differentiation in an *ex vivo* environment.

Since targeting the endogenous regenerative process is a conceptually attractive approach to enhance remyelination, the lysolecithin model of demyelination has also been widely used to investigate the remyelinating potential of exogenous drugs as well as antagonists/agonists of receptors and proteins involved in OP differentiation. In this regard, in a recent study we have

shown that selective Tumor Necrosis Factor Receptor-2 activation by specific agonistic antibody enhances oligodendrocyte maturation and myelin protein expression and promotes a neuroprotective milieu in mouse cerebellar slices following toxin-induced myelin damage (Veroni *et al.*, 2020).

In addition, by acting as an important link between high-throughput *in vitro* approaches and animal models, lysolecithin treated slices have been also used in several drug repurposing studies, which hold great promise in the field of demyelinating diseases, leading to the identification of several modulators of remyelination (Cole *et al.*, 2017). In 2017, we performed an extensive screening of a library of 2000 drugs and other natural substances to test their remyelinating potential using *in vitro* models and validating the most promising compounds in organotypic cerebellar slice models of both myelination and demyelination/remyelination; we identified two molecules able to promote remyelination, namely the radical scavenger edaravone and the 5-methyl-7-methoxyisoflavone (Eleuteri *et al.*, 2017). These models may thus represent powerful approaches in drug screening studies, avoiding the use of animal models for the validation of a large number of compounds, showing sufficient throughput and enough biological complexity to allow pre-selection of compounds for further *in vivo* studies.

References

Ajmone-Cat MA, D'Urso MC, di Blasio G, Brignone MS, De Simone R, Minghetti L. Glycogen synthase kinase 3 is part of the molecular machinery regulating the adaptive response to LPS stimulation in microglial cells. *Brain Behav Immun* 2016;55:225–35.

Ajmone-Cat MA, Mancini M, De Simone R, Cilli P, Minghetti L. Microglial polarization and plasticity: evidence from organotypic hippocampal slice cultures. *Glia* 2013;61(10):1698-711.

Ajmone-Cat MA, Nicolini A, Minghetti L. Prolonged exposure of microglia to lipopolysaccharide modifies the intracellular signaling pathways and selectively promotes prostaglandin E2 synthesis. *J Neurochem* 2003;87(5):1193-203.

Baudouin L, Adès N, Kanté K, Czarnecki A, Bachelin C, Baskaran A, Langui D, Millécamps A, Gurchenkov B, Velut Y, Duarte K, Barnier JV, Nait Oumesmar B, Bouslama-Oueghlani L. Co-culture of exogenous oligodendrocytes with unmyelinated cerebella: Revisiting *ex vivo* models and new tools to study myelination. *Glia* 2021;69(8):1916-31.

Cacci E, Ajmone-Cat MA, Anelli T, Biagioni S, Minghetti L. *In vitro* neuronal and glial differentiation from embryonic or adult neural precursor cells are differently affected by chronic or acute activation of microglia. *Glia* 2008;56(4):412-25.

Cole KLH, Early JJ, Lyons DA. Drug discovery for remyelination and treatment of MS. *Glia* 2017;65(10):1565-89.

Coleman LG Jr, Zou J, Crews FT. Microglial depletion and repopulation in brain slice culture normalizes sensitized proinflammatory signaling. *J Neuroinflammation* 2020;17(1):27.

Cunniffe N, Coles A. Promoting remyelination in multiple sclerosis. *J Neurol* 2021;26(1):30-44.

de Billy E, Pellegrino M, Orlando D, Pericoli G, Ferretti R, Businaro P, Ajmone-Cat MA, Rossi S, Petrilli LL, Maestro N, Diomedi-Camassei F, Pezzullo M, De Stefanis C, Bencivenga P, Palma A, Rota R, Del Bufalo F, Massimi L, Weber G, Jones C, Carai A, Caruso S, De Angelis B, Caruana I, Quintarelli C, Mastronuzzi A, Locatelli F, Vinci M. Dual IGF1R/IR inhibitors in combination with GD2-CAR T-cells display a potent anti-tumor activity in diffuse midline glioma H3K27M-mutant. *Neuro Oncol* 2021:noab300. doi: 10.1093/neuonc/noab300.

Doussau F, Dupont JL, Neel D, Schneider A, Poulain B, Bossu JL. Organotypic cultures of cerebellar slices as a model to investigate demyelinating disorders. *Expert Opin Drug Discov* 2017;12(10):1011-22.

Eleuteri C, Olla S, Veroni C, Umeton R, Mechelli R, Romano S, Buscarinu MC, Ferrari F, Calò G, Ristori. G, Salvetti M, Agresti C. A staged screening of registered drugs highlights remyelinating drug candidates for clinical trials. *Sci Rep* 2017;7:45780.

Franklin RJM, Frisen J, Lyons DA. Revisiting remyelination: towards a consensus on the regeneration of CNS myelin. *Semin Cell Dev Biol* 2021;116:3-9.

Gilhus NE, Deuschl G. Neuroinflammation - a common thread in neurological disorders. *Nat Rev Neurol* 2019;15(8):429-30.

Humpel C. Organotypic Brain Slices of ADULT Transgenic Mice: a tool to study Alzheimer's disease. *Curr Alzheimer Res* 2019;16(2):172-81.

Neher J.J. and Cunningham C. Priming Microglia for Innate Immune Memory in the Brain. *Trends in Immunology* 2019;40(4):358-74.

Pericoli G, Petrini S, Giorda E, Ferretti R, Ajmone-Cat MA, Court W, Conti LA, De Simone R, Bencivenga P, Palma A, Di Giannatale A, Jones C, Carai A, Mastronuzzi A, de Billy E, Locatelli F, Vinci M. Integration of multiple platforms for the analysis of multifluorescent marking technology applied to pediatric GBM and DIPG. *Int J Mol Sci.* 2020;21(18):6763.

Shamir E, Ewald A. Three-dimensional organotypic culture: experimental models of mammalian biology and disease. *Nat Rev Mol Cell Biol* 15:647-64.

Veroni C, Serafini B, Rosicarelli B, Fagnani C, Aloisi F, Agresti C. Connecting immune cell infiltration to the multitasking microglia response and TNF receptor 2 induction in the multiple sclerosis brain. *Front Cell Neurosci* 2020;14:190.

Vinci M, Burford A, Molinari V, Kessler K, Popov S, Clarke M, Taylor KR, Pemberton HN, Lord CJ, Gutteridge A, *et al.* Functional diversity and cooperativity between subclonal populations of pediatric glioblastoma and diffuse intrinsic pontine glioma cells. *Nat Med* 2018;24:1204-15.

Zhang J, Kramer EG, Mahase S, Dutta DJ, Bonnamain V, Argaw AT, John GR. Targeting oligodendrocyte protection and remyelination in multiple sclerosis. *Mt Sinai J Med.* 2011;78(2):244-57.