Synopsis of the thesis on

Role of astrocytic MeCP2 in the regulation of central nervous system myelination

To be submitted to

The Maharaja Sayajirao University of Baroda

For the degree of

Doctor of Philosophy in Zoology

By

Lipi Buch

Under supervision of

Dr. Prakash P. Pillai
Division of Neurobiology

Department of Zoology
Faculty of Science
The Maharaja Sayajirao University of Baroda
Vadodara- 390 002

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INTRODUCTION

In all complex nervous systems neuronal cells coexist with glial cells. This suggests that neuron-glia interactions are principle features for proper functioning of both types of cells. One principle neuro-glia interaction is myelination- process by which an axon becomes insulated by the continuous wrapping of the lipid rich glial membrane known as myelin. In PNS, Schwann cells perform the function of myelination while oligodendrocytes are the myelinating cells of the CNS. Myelination occurs through a number of sequential steps: migration of oligodendrocyte precursor cells (OPCs) along the white matter tracks, adhesion of the oligodendrocyte process to the axon, spiralling of the process around the axon, compaction of the myelin sheath (Nave & Trapp, 2008; Nave & Werner, 2014). The need for myelination occurs to support the effective transduction of action potentials via saltatory impulse propagation and also providing an additional trophic and metabolic support to the axons.

Myelination depends on a number of intrinsic transcription factors like Olig1, YY1, Sox10, signaling pathways like integrin, neuregulin, notch signaling and extrinsic factors like trophic factors such as neurotrophins, growth factors, electrical activity from neurons and the myelinating milieu supported by a number of secreted factors from astrocytes. Moreover, cell-cell interaction between neurons and oligodendrocytes also known as axo-glial interactions, is another key factor influencing myelination.

Astrocytes and Myelination: Astrocytes are important in assisting CNS myelination (Barnett & Linington, 2013; Kıray et al., 2016; Williams et al., 2007). Studies have demonstrated that astrocytes regulate CNS myelination by secreting factors which promote or inhibit myelination. Some of these factors are: LIF (Ishibashi et al., 2006), γ-secretase (Sorensen et al., 2008; Watkins et al., 2008), CXCL10 (Nash et al., 2011), PDGF (Bögler et al., 1990), TIMP-1 (Moore et al., 2011), CNTF (Nash et al., 2011), neurotrophins like BDNF (Xiao et al., 2010), NGF (Yin et al., 2012), which drive the myelination event. Presence of astrocytes in myelinating cultures also increase the speed

of myelin wrapping around axons (Watkins et al., 2008). The crucial role of astrocytes in myelination is evident by the failure to replicate the complex cellular environment of myelination in vivo in their absence (Talbott et al., 2005).

Axo-glial junctions and astrocytes: The ability of myelinated axons to conduct saltatory conductions depends on the distribution of numerous molecular components into distinct domains- the internode, the paranode and the node of Ranvier which form as the result of specific interactions between axons and myelinating cells (Devaux & Faivre-Sarrailh, 2013). The first molecular constituent of the nodal junctions is the axonal contactin-associated protein, Caspr or Paranodin (Einheber et al., 1997) the glial ligand for which is the neurofascin isoform NF155, which is expressed by myelinating glia in the paranodal region (Tait et al., 2000). In addition other molecules- namely Notch1 (Hu et al., 2004), Neuregulin (Canoll et al., 1996) and NCAM (Charles et al., 2000) have been reported to be expressed at the site of axo-glial interactions. A special class of astrocytes extends through the nodal gap substance and approaches the node where it becomes closely associated with the nodal part of the axon membrane (Araque et al., 1999; Perez-Alvarez & Araque, 2013). They secrete extracellular matrix protein cytotactin (Rieger et al., 1986) which functions in electrical coupling of extracellular ions during the conduction of action potentials, clearing extracellular potassium released by neuronal activity (Serwanski et al., 2017; Waxman & Quick, 1978) and buffering the perinodal space (Gutnick et al., 1981; Masa & Mugnaini, 1982). Thus, the assembly and/or maintenance of nodal membrane may be indirectly related to the presence of perinodal astrocyte processes (Black & Waxman, 1988).

Astrocytes and Neuronal interactions: The ratio of astrocytes to neurons varies among species, increasing proportionally with the complexity of the neural network (Banaclocha, 2007). Astrocytes have been long known support and promote neuronal survival and neurite formation through secretion of various trophic factors in the neuronal surroundings including NGF, BDNF, LIF, FGF-2 and CNTF (Albrecht et al., 2007; Drukarch et al., 1998; Messersmith et al., 2000; Schwartz & Nishiyama, 1994). They are also found to be closely associated with axons at an early stage of their myelination by oligodendrocytes (Ioannidou et al., 2014). An important role of astrocytes is to act as a

regulator of synaptic transmission via gliotransmitter release which coordinates neuronal activity through dynamic communication between the astrocytic process and the pre- and postsynaptic terminals (Halassa et al., 2009). Thus astrocytes are in direct communication with neurons, modulating their function and ultimately providing a significant impact on physiological and pathological conditions.

Astrocytes and Oligodendrocytes' interactions: Astrocytes function in close proximity with oligodendrocytes and are involved in their development and biogenesis. They induce oligodendrocytes to align their processes with axons (Meyer-Franke et al., 1999). Astrocytes release ATP, adenosine and neurotransmitters that elicit transient increase of cytosolic Ca2+ which in turn regulates almost all cellular processes in oligodendrogial differentiation (Butt, 2006; Ishibashi et al., 2006). Certain cytokines like LIF (Biancotti et al., 2008; Ishibashi et al., 2009; Nair et al., 2008) and neurotrophins like NGF and BDNF (Miyamoto et al., 2015) are also secreted by astrocytes which are crucial for timing oligodendrogenesis, development and function. Proximity of astrocytes enhances and remodels the myelination substrate of the neighboring oligodendrocytes non-cell autonomously (Iacobas & Iacobas, 2010). Myelin membrane formation by oligodendrocytes also requires extracellular lipids provided by astrocytes (Camargo et al., 2017).

MeCP2

MeCP2 is a member of a family of DNA-binding proteins and a genome-wide, transcriptional modulator involved in many CNS functions. MeCP2 is reported to be the key molecule involved in the neurodevelopmental disease, Rett syndrome characterized by loss of motor skills, microcephaly, mental retardation and autism (Amir et al., 1999). It is found in most tissues and cell types in the body but with highest expression levels in the brain especially in post migratory neurons. MeCP2 is reported to interact with some master transcriptional regulators such as REST and coREST, in regulating neuronal and glial cell specificity (Ballas et al., 2005). These REST and coREST, in turn regulate Itgb1 (Integrin beta 1 precursor- an integrin required to maintain oligodendrocyte-axonal

contact during myelination), ID4 (factor differentially regulating myelination by altering myelin proteins), Gpr158 and many more. REST and coREST also have diverse functions in modulating several signalling pathways such as Notch, Neuregulin and Integrin signalling which mediates cell-cell interactions (Abrajano et al., 2009).

MeCP2 deficiency is known to cause microcephaly in major brain regions, delayed neuronal maturation and synaptogenesis, reduced dendritic spine density, dendritic branching, abnormal number of axons and a defect in axonal targeting (Guy et al., 2011). Neural maturation and synapse formation correlate with MeCP2 protein levels which increases as the cells mature (Kaufmann et al., 2005). Recently, expression of MeCP2 has been reported in glial cells- oligodendrocytes, astrocytes and microglia. MeCP2-deficient oligodendrocytes differentially express myelin specific proteins (Sharma et al., 2015) whereas MeCP2-deficient microglia cause dendritic and synaptic damage mediated by elevated glutamate release (Maezawa & Jin, 2010).

Astrocytes and MeCP2: Recently presence of MeCP2 was reported in astrocytes at a five-fold lower level than in neurons. Its deficiency causes neuronal morphological abnormalities like reduced dendritic outgrowth, disruption of support for neuronal dendritic maturation and causes abnormal dendritic arborisation (Ballas et al., 2009; Maezawa et al., 2009). In addition, it was studied that MeCP2 negative astrocytes exert a non-cell autonomous effect that negatively influence MeCP2 levels of normal astrocytes; this effect is partially mediated by gap junction communication. Astrocytes from RTT male mice, as well as their conditioned medium, cause aberrant dendritic morphology in WT hippocampal neurons. Whereas, re-expression of MeCP2 in astrocytes significantly improves locomotion, anxiety levels and average lifespan, suggesting that astrocyte dysfunction is involved in the neuropathology and characteristic phenotypic regression of RTT (Maezawa et al., 2009). Loss of MeCP2 from astrocytes also causes dysregulation of genes of tripartite synapse like Cntn1, Syn2, Gabrg1 and Gria1; which could affect the ability of astrocytes to sense neuronal activity and to modulate synaptic transmission (Yasui et al., 2013).

In view of all the above reports, we hypothesized that astrocytic MeCP2 have a pivotal role in oligodendrocyte and neuronal physiology by secreting some essential factors along with regulation of axo-glial interactions and myelination related events.

In the present study, we focused on the effect of MeCP2 on molecules secreted by astrocytes which have been known to regulate myelination, its effect on oligodendrocyte and neuronal survival and proliferation, in regulation of axo-glial assembly molecules like Caspr, NF155, Nrg1, Notch receptors and finally on the overall effect of deficient astrocytic MeCP2 on key myelination genes.

Findings from the current study enhances the existing understanding on the involvement of astrocytic MeCP2 in myelination which will help to understand several brain disorders characterized by dysfunctional myelination.

Objectives:

1. To determine the role of MeCP2 on astrocyte secreted factors regulating myelination.
2. To determine the effect of MeCP2 deficient astrocytes on oligodendrocytes and neuronal survival, proliferation and differentiation.
3. To study the effect of astrocytic MeCP2 in mapping of axo-glial interactions and events of myelination.

1. To determine the role of MeCP2 on astrocyte secreted factors regulating myelination

In the present study, we hypothesized that MeCP2 regulates certain astrocytic genes that are known to influence myelination. We transfected astrocytes with specific MeCP2 siRNA to induce a 40-50% silencing of MeCP2 and analysed certain astrocytic genes- CNTF, LIF, PDGFR and CXCL10. It was found that levels of CNTF and LIF (myelination promoters) were inhibited and those of PDGFR and CXCL10 (myelination inhibitors) were elevated in response to MeCP2 deficiency in astrocytes. Also, secreted levels of BDNF, a key neurotrophin involved in CNS myelination were found to be decreased in MeCP2 knockdown astrocyte condition media in spite of elevated levels of

BDNF in astrocyte protein pool thus suggesting role of MeCP2 in affecting the secretory pathway of BDNF in astrocytes.

2. To determine the effect of MeCP2 deficient astrocytes on oligodendrocytes and neuronal survival, proliferation and differentiation.

We studied the effect of MeCP2 deficient astrocytes on oligodendroglial survival and proliferation for which oligodendrocyte precursor cells (OPCs) were grown in MeCP2 knockdown astrocyte condition media (MkACM). Results indicate decreased cell viability in OPCs grown in MkACM compared to DMEM control indicating contribution of astrocytes in oligodendrocyte survival possibly by secreting some cell soluble factors. Moreover, the proliferation capacity of OPCs was also compromised in presence of MkACM compared to control. An interesting observation was in the form of oligodendrocyte morphological studies where Myelin Basic Protein (MBP) fluorescence levels were found to be elevated along with its extensive branching morphology in MkACM treated oligodendrocytes. Astrocytic MeCP2 is also found to regulate neuronal growth and survival. Neuronal morphology showed shorter and aberrant dendritic process morphology when cultured in MkACM, an observation concurrent with transcript and protein expression of neuronal marker, Tuj1 which was down-regulated in neurons treated with MkACM, in addition to regulating the cell survival of neurons.

3. To study the effect of astrocytic MeCP2 in mapping of axo-glial interactions and events of myelination.

To study the role of MeCP2 in regulation of axo-glial interactions, some key axo-glial molecules were identified and their transcript levels were analysed in MeCP2 deficient oligodendrocytes and MeCP2 deficient DRG neurons. NF155 and Notch are axo-glial genes which are known to be expressed on oligodendroglial cell membrane during the initial phases of myelination. We observed down regulation of NF155 gene in MeCP2 knockdown oligodendrocytes while Notch was up-regulated but not significantly. We then assessed the expression of Caspr and Nrg1- axo-glial genes known to be expressed on neuronal membrane and observed significant up-regulation of both Caspr and Nrg1 in MeCP2 knockdown DRG neurons which indicates regulation of these genes by DRG MeCP2.

To assess the effect of astrocytic MeCP2 on axo-glial interaction genes, co-cultures of DRG neurons and oligodendrocytes were plated on wild type and MeCP2 siRNA transfected astrocytes followed by analysing the transcript levels of axo-glial interaction genes- NF155, Notch, Caspr and Nrg1. Transcript levels of oligodendrocyte specific axo-glial genes- NF155 and Notch were not regulated whereas neuronal specific axo-glial genes- Caspr and Nrg1 were significantly up-regulated by astrocytic MeCP2 compared to co-cultures with wild-type astrocytes.

Primary cell cultures of oligodendrocytes, astrocytes and DRG neuronal cultures were successfully established, maintained and characterized. Co-culture of oligodendrocytes and DRG neurons was also successfully established and characterized identified using cell specific markers, Myelin Basic Protein (MBP) and Neurofilament (NF), respectively. Upon closer examination, oligodendrocytes are evidenced to make contact with numerous DRG Neurons, often ensheathing them with an MBP+ membrane thus confirming myelination. To assess the effect of astrocytic MeCP2 on myelin genes, co-cultures of DRG neurons and Oligodendrocytes were plated on wild type and MeCP2 siRNA transfected astrocytes followed by analyzing the transcript levels of myelin genes by real time PCR. Transcript levels of myelin genes– Myelin Basic Protein (MBP), Proteolipid protein (PLP), Myelin Associated Glycoprotein (MAG) and Myelin oligodendrocyte glycoprotein (MOG) were found to be decreased in MeCP2 knockdown astrocytes with DRG neurons & oligodendrocytes compared to control suggesting a key role of astrocytic MeCP2 in regulation of myelination.

Conclusion:

Present study demonstrates that MeCP2 regulates certain astrocytic genes and neurotrophins involved in CNS myelination along with oligodendrocyte and neuronal morphology, survival and proliferation. Moreover, MeCP2 in oligodendrocytes and DRG neurons regulate certain axo-glial interactions genes thus being involved in the initiation of myelination. Astrocytic MeCP2 is also shown to be involved in CNS myelination by regulating axo-glial interaction genes along with key myelin genes.

The present study, to the best of current knowledge is the first report which states the role of astrocytic MeCP2 in axo-glial interactions and CNS myelination.

Acknowledgements:

This work was supported by grant from the Department of Biotechnology (DBT), New Delhi, India, (sanction order no. BT/PR4974/MED/30/773/2012 dated 10/09/2013). We thank the Department of Biotechnology (DBT), New Delhi, India for awarding Junior Research Fellowship (JRF) and Senior Research Fellowship (SRF) to Lipi Buch. We are also very thankful to DBT-ILSPARE Programme, for the confocal microscopy and real-time PCR facility at Dr. Vikram Sarabhai Science Block, Faculty of Science, MSU, Baroda.

Animal Ethical Statement: All animal protocols were approved by the Institutional Animal Ethical Committee, The Maharaja Sayajirao University of Baroda, Department of Biochemistry; (ZD/13/2014, ZD/31/2014, ZD/01/2016).

References:

Abrajano, J. J., Qureshi, I. A., Gokhan, S., Zheng, D., Bergman, A., & Mehler, M. F. (2009). REST and CoREST modulate neuronal subtype specification, maturation and maintenance. *PloS one*, *4*(12), e7936.

Albrecht, P. J., Enterline, J. C., Cromer, J., & Levison, S. W. (2007). CNTF-activated astrocytes release a soluble trophic activity for oligodendrocyte progenitors. *Neurochemical research*, *32*(2), 263-271.

Amir, R. E., Van den Veyver, I. B., Wan, M., Tran, C. Q., Francke, U., & Zoghbi, H. Y. (1999). Rett syndrome is caused by mutations in X-linked MECP2, encoding methyl-CpG-binding protein 2. *Nature genetics*, *23*(2), 185-188.

Araque, A., Parpura, V., Sanzgiri, R. P., & Haydon, P. G. (1999). Tripartite synapses: glia, the unacknowledged partner. *Trends in neurosciences*, *22*(5), 208-215.

Ballas, N., Grunseich, C., Lu, D. D., Speh, J. C., & Mandel, G. (2005). REST and its corepressors mediate plasticity of neuronal gene chromatin throughout neurogenesis. *Cell*, *121*(4), 645-657.

Ballas, N., Lioy, D. T., Grunseich, C., & Mandel, G. (2009). Non–cell autonomous influence of MeCP2-deficient glia on neuronal dendritic morphology. *Nature neuroscience*, *12*(3), 311-317.

Banaclocha, M. A. M. (2007). Neuromagnetic dialogue between neuronal minicolumns and astroglial network: A new approach for memory and cerebral computation. *Brain research bulletin*, *73*(1), 21-27.

Barnett, S. C., & Linington, C. (2013). Myelination: do astrocytes play a role? *The Neuroscientist*, *19*(5), 442-450.

Biancotti, J. C., Kumar, S., & de Vellis, J. (2008). Activation of inflammatory response by a combination of growth factors in cuprizone-induced demyelinated brain leads to myelin repair. *Neurochemical research*, *33*(12), 2615-2628.

Black, J. A., & Waxman, S. G. (1988). The perinodal astrocyte. *Glia*, *1*(3), 169-183.

Bögler, O., Wren, D., Barnett, S. C., Land, H., & Noble, M. (1990). Cooperation between two growth factors promotes extended self-renewal and inhibits differentiation of oligodendrocyte-type-2 astrocyte (O-2A) progenitor cells. *Proceedings of the National Academy of Sciences, 87*(16), 6368-6372.

Butt, A. M. (2006). Neurotransmitter-mediated calcium signalling in oligodendrocyte physiology and pathology. *Glia, 54*(7), 666-675.

Camargo, N., Goudriaan, A., van Deijk, A.-L. F., Otte, W. M., Brouwers, J. F., Lodder, H., . . . Mansvelder, H. D. (2017). Oligodendroglial myelination requires astrocyte-derived lipids. *PLoS biology, 15*(5), e1002605.

Canoll, P. D., Musacchio, J., Hardy, R., Reynolds, R., Marchionni, M. A., & Salzer, J. L. (1996). GGF/neuregulin is a neuronal signal that promotes the proliferation and survival and inhibits the differentiation of oligodendrocyte progenitors. *Neuron, 17*(2), 229-243.

Charles, P., Hernandez, M., Stankoff, B., Aigrot, M., Colin, C., Rougon, G., . . . Lubetzki, C. (2000). Negative regulation of central nervous system myelination by polysialylated-neural cell adhesion molecule. *Proceedings of the National Academy of Sciences, 97*(13), 7585-7590.

Devaux, J. J., & Faivre-Sarrailh, C. (2013). Neuro-glial interactions at the nodes of Ranvier: implication in health and diseases. *Frontiers in cellular neuroscience, 7*, 196.

Drukarch, B., Schepens, E., Stoof, J. C., Langeveld, C. H., & Van Muiswinkel, F. L. (1998). Astrocyte-enhanced neuronal survival is mediated by scavenging of extracellular reactive oxygen species. *Free Radical Biology and Medicine, 25*(2), 217-220.

Einheber, S., Zanazzi, G., Ching, W., Scherer, S., Milner, T. A., Peles, E., & Salzer, J. L. (1997). The axonal membrane protein Caspr, a homologue of neurexin IV, is a component of the septate-like paranodal junctions that assemble during myelination. *The Journal of cell biology, 139*(6), 1495-1506.

Gutnick, M. J., Connors, B. W., & Ransom, B. R. (1981). Dye-coupling between glial cells in the guinea pig neocortical slice. *Brain research, 213*(2), 486-492.

Guy, J., Cheval, H., Selfridge, J., & Bird, A. (2011). The role of MeCP2 in the brain. *Annual review of cell and developmental biology, 27*, 631-652.

Halassa, M. M., Fellin, T., & Haydon, P. G. (2009). Tripartite synapses: roles for astrocytic purines in the control of synaptic physiology and behavior. *Neuropharmacology, 57*(4), 343-346.

Hu, Q., Cui, X., Ng, Y., & Xiao, Z. (2004). Axoglial interaction via the notch receptor in oligodendrocyte differentiation. *ANNALS-ACADEMY OF MEDICINE SINGAPORE, 33*(5), 581-588.

Iacobas, S., & Iacobas, D. A. (2010). Astrocyte proximity modulates the myelination gene fabric of oligodendrocytes. *Neuron glia biology, 6*(3), 157-169.

Ioannidou, K., Anderson, K. I., Strachan, D., Edgar, J. M., & Barnett, S. C. (2014). Astroglial-axonal interactions during early stages of myelination in mixed cultures using in vitro and ex vivo imaging techniques. *BMC neuroscience, 15*(1), 59.

Ishibashi, T., Dakin, K. A., Stevens, B., Lee, P. R., Kozlov, S. V., Stewart, C. L., & Fields, R. D. (2006). Astrocytes promote myelination in response to electrical impulses. *Neuron, 49*(6), 823-832.

Ishibashi, T., Lee, P. R., Baba, H., & Fields, R. D. (2009). Leukemia inhibitory factor regulates the timing of oligodendrocyte development and myelination in the postnatal optic nerve. *Journal of neuroscience research, 87*(15), 3343-3355.

Kaufmann, W. E., Johnston, M. V., & Blue, M. E. (2005). MeCP2 expression and function during brain development: implications for Rett syndrome's pathogenesis and clinical evolution. *Brain and Development, 27*, S77-S87.

Kıray, H., Lindsay, S. L., Hosseinzadeh, S., & Barnett, S. C. (2016). The multifaceted role of astrocytes in regulating myelination. *Experimental neurology, 283*, 541-549.

Maezawa, I., & Jin, L.-W. (2010). Rett syndrome microglia damage dendrites and synapses by the elevated release of glutamate. *Journal of Neuroscience, 30*(15), 5346-5356.

Maezawa, I., Swanberg, S., Harvey, D., LaSalle, J. M., & Jin, L.-W. (2009). Rett syndrome astrocytes are abnormal and spread MeCP2 deficiency through gap junctions. *Journal of Neuroscience, 29*(16), 5051-5061.

Masa, P., & Mugnaini, E. (1982). Cell junctions and intramembrane particles of astrocytes and oligodendrocytes: a freeze-fracture study. *Neuroscience, 7*(2), 523-538.

Messersmith, D. J., Murtie, J. C., Le, T. Q., Frost, E. E., & Armstrong, R. C. (2000). Fibroblast growth factor 2 (FGF2) and FGF receptor expression in an experimental demyelinating disease with extensive remyelination. *Journal of neuroscience research, 62*(2), 241-256.

Meyer-Franke, A., Shen, S., & Barres, B. A. (1999). Astrocytes induce oligodendrocyte processes to align with and adhere to axons. *Molecular and Cellular Neuroscience, 14*(4), 385-397.

Miyamoto, N., Maki, T., Shindo, A., Liang, A. C., Maeda, M., Egawa, N., . . . Ihara, M. (2015). Astrocytes promote oligodendrogenesis after white matter damage via brain-derived neurotrophic factor. *Journal of Neuroscience, 35*(41), 14002-14008.

Moore, C. S., Abdullah, S. L., Brown, A., Arulpragasam, A., & Crocker, S. J. (2011). How factors secreted from astrocytes impact myelin repair. *Journal of neuroscience research, 89*(1), 13-21.

Nair, A., Frederick, T. J., & Miller, S. D. (2008). Astrocytes in multiple sclerosis: a product of their environment. *Cellular and molecular life sciences, 65*(17), 2702.

Nash, B., Thomson, C. E., Linington, C., Arthur, A. T., McClure, J. D., McBride, M. W., & Barnett, S. C. (2011). Functional duality of astrocytes in myelination. *Journal of Neuroscience, 31*(37), 13028-13038.

Nave, K.-A., & Trapp, B. D. (2008). Axon-glial signaling and the glial support of axon function. *Annu. Rev. Neurosci., 31*, 535-561.

Nave, K.-A., & Werner, H. B. (2014). Myelination of the nervous system: mechanisms and functions. *Annual review of cell and developmental biology, 30*, 503-533.

Perez-Alvarez, A., & Araque, A. (2013). Astrocyte-neuron interaction at tripartite synapses. *Current drug targets, 14*(11), 1220-1224.

Rieger, F., Daniloff, J. K., Pincon-Raymond, M., Crossin, K. L., Grumet, M., & Edelman, G. M. (1986). Neuronal cell adhesion molecules and cytotactin are colocalized at the node of Ranvier. *The Journal of cell biology, 103*(2), 379-391.

Schwartz, J. P., & Nishiyama, N. (1994). Neurotrophic factor gene expression in astrocytes during development and following injury. *Brain research bulletin, 35*(5), 403-407.

Serwanski, D. R., Jukkola, P., & Nishiyama, A. (2017). Heterogeneity of astrocyte and NG2 cell insertion at the node of ranvier. *Journal of Comparative Neurology, 525*(3), 535-552.

Sharma, K., Singh, J., Pillai, P. P., & Frost, E. E. (2015). Involvement of MeCP2 in regulation of myelin-related gene expression in cultured rat oligodendrocytes. *Journal of Molecular Neuroscience, 57*(2), 176-184.

Sorensen, A., Moffat, K., Thomson, C., & Barnett, S. C. (2008). Astrocytes, but not olfactory ensheathing cells or Schwann cells, promote myelination of CNS axons in vitro. *Glia, 56*(7), 750-763.

Tait, S., Gunn-Moore, F., Collinson, J. M., Huang, J., Lubetzki, C., Pedraza, L., . . . Brophy, P. J. (2000). An oligodendrocyte cell adhesion molecule at the site of assembly of the paranodal axo-glial junction. *The Journal of cell biology, 150*(3), 657-666.

Talbott, J. F., Loy, D. N., Liu, Y., Qiu, M. S., Bunge, M. B., Rao, M. S., & Whittemore, S. R. (2005). Endogenous Nkx2. 2+/Olig2+ oligodendrocyte precursor cells fail to remyelinate the demyelinated adult rat spinal cord in the absence of astrocytes. *Experimental neurology, 192*(1), 11-24.

Watkins, T. A., Emery, B., Mulinyawe, S., & Barres, B. A. (2008). Distinct stages of myelination regulated by γ-secretase and astrocytes in a rapidly myelinating CNS coculture system. *Neuron, 60*(4), 555-569.

Waxman, S. G., & Quick, D. C. (1978). Intra-axonal ferric ion-ferrocyanide staining of nodes of Ranvier and initial segments in central myelinated fibers. *Brain research, 144*(1), 1-10.

Williams, A., Piaton, G., & Lubetzki, C. (2007). Astrocytes—friends or foes in multiple sclerosis? *Glia, 55*(13), 1300-1312.

Xiao, J., Wong, A. W., Willingham, M. M., van den Buuse, M., Kilpatrick, T. J., & Murray, S. S. (2010). Brain-derived neurotrophic factor promotes central nervous system myelination via a direct effect upon oligodendrocytes. *Neurosignals, 18*(3), 186-202.

Yasui, D. H., Xu, H., Dunaway, K. W., LaSalle, J. M., Jin, L.-W., & Maezawa, I. (2013). MeCP2 modulates gene expression pathways in astrocytes. *Molecular autism, 4*(1), 3.

Yin, L.-L., Lin, L.-L., Zhang, L., & Li, L. (2012). Epimedium flavonoids ameliorate experimental autoimmune encephalomyelitis in rats by modulating neuroinflammatory and neurotrophic responses. *Neuropharmacology, 63*(5), 851-862.

Workshops/conferences/seminars:

- Poster presentation entitled "Role of Methyl-CpG-Binding-Protein 2 (MeCP2) in impaired mitochondrial functions in glial cells" presented at the 28th Annual Meeting of Society For Neurochemistry, India (SNCI), September 3rd-12th, 2014, Chennai, T.N (Co-author, Presenting Author)
- Poster presentation entitled "Role of Astroglial Methyl-CpG-Binding Protein 2 in Central Nervous System Myelination" presented at the 25th Biennial meeting of the International Society of Neurochemistry (ISN) and Asian Pacific Society of Neurochemistry (APSN), Cairns, Australia, August 23rd-27th, 2015 (First Author)
- Poster presentation entitled "Role of astrocytic MeCP2 in the regulation of central nervous system myelination" presented at the 34th Annual Meeting of Indian Academy of Neurosciences, National Brain Research Centre, Manesar, India, October 19th-21st, 2016 (First author)

Publications:

1. Pillai, V., Kadu, R., Buch, L., & Singh, V. K. (2017). Derivatives of Dapsone (dap): Synthesis and Study on In Vitro Anticancer Activity and DNA Laddering Against Hep G2 and C6 Human Cancer Cell Lines. ChemistrySelect, 2(16), 4382-4391.
2. Pillai, V., Buch, L., Desai, A., & Singh, V. K. (2017). Derivatives of 4, 4'-Oxydianiline Show Distinct in Vitro Cytotoxicity, Apoptosis Induction, and Selectivity against HepG2 Cancer Cells. ChemistrySelect, 2(35), 11581-11589.
3. L Buch, J Langhnoja, P Pillai. 2015/8/1. "Role of astroglial Methyl-CpG-binding protein 2 in central nervous system myelination." Journal of Neurochemistry, Volume 134, Pages 283-283, Publisher WILEYBLACKWELL.
4. J Langhnoja, L Buch, P Pillai. 2015/8/1. "Modulatory effects of tumor necrosis factor α on neurotrophins in myelinating spinal cord and dorsal root ganglion cultures." Journal of Neurochemistry, Volume 134, Pages 201-201, Publisher WILEYBLACKWELL.
5. L Buch, J Langhnoja, P Pillai, 2017, "Neurotrophomodulatory effect of TNF-α in rat cortical astrocytes through NF-κB pathway" (Under review).
6. L Buch, J Langhnoja, P Pillai, 2017, "Role of astrocytic MeCP2 in regulation of CNS myelination by affecting oligodendrocyte and neuronal physiology and axo-glial interactions" (Under communication).
7. J Langhnoja, L Buch, P Pillai, 2017, "17-β Estradiol modulates NGF and BDNF expression through ERβ mediated ERK signalling in cortical astrocytes" (Under review).