NEUROPATHOLOGICAL EXAMINATIONS IN THE MOUSE BRAIN- INVESTIGATION OF A NEW PHARMACEUTICAL TARGET IN THE CUPRIZONE MODEL AND COMPARATIVE HISTOLOGICAL ANALYSES OF PRIMARY CILIA IN THE PHYSIOLOGICAL CENTRAL NERVOUS SYSTEM

Ph.D. Thesis

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1. INTRODUCTION

1.1 General overview and pathology of Multiple Sclerosis

Multiple Sclerosis (MS) is a chronic and degenerative disease of the central nervous system (CNS) characterised by inflammation and myelin destruction. MS has been one of the leading causes of neurological disability among young adults. The initial symptoms or onset of the disease is typically diagnosed between the age of 25-35. It predominantly affects the women gender (female to male ratio varies between 1,5:1 and 2,5:1) [**1**], and generally found to be more prevalent in countries of the Northern hemisphere [**2**]. Clinically, MS is a heterogeneous disease and has a great diversity in the presentation of neurological symptoms across individuals. Depending on the affected CNS region- the brain and the spinal cord- the clinical manifestations may arise as visual disturbances, widespread dysfunctions of the motor and autonome system, sensory abnormalities or even emotional and cognitive changes [**3-12**]. The combination and intensity of symptoms vary from individual to individual and may appear in different repetitive timeframes throughout different disease courses. Most patients experience temporary neurological disabilities followed by partial or complete recovery (relapsing-remitting form of MS or RRMS). However, the frequency of symptoms usually increase by time and become permanent in later stages of MS (secondary progressive form of MS or SPMS). Some patients suffer a progressive neurological decline from onset of the disease (primary progressive from of MS or PPMS). In rare cases, progressive accumulation of neurological disability is present from onset and also associated with acute relapses with or without remission (progressive-relapsing form of MS or PRMS) [**4, 13-16**]. The complex nature of MS has been extensively studied throughout decades. Both genetic and environmental factors are well-established components of disease susceptibility [**1**]. However, the exact pathomechanism of MS is still elusive and even separate pathological processes have been suggested to underlie the distinct clinical courses [**17, 18**].

The neuropathological hallmark of MS is the presence of focal demyelinating plaque-like lesions characterised by oligodendrocyte (OL) destruction, inflammation, axonal injury and reactive gliosis [**3, 19, 20**]. Traditionally, lesion genesis is thought to occur on an inflammatory background in which activated, self-reactive T lymphocytes cross a disintegrated blood-brain-barrier (BBB), sequentially activate local, residential immune cells and propagate an inflammatory destruction of oligodendrocytes (OL) and myelin [**19, 21**]. However, the immunopathological appearance of MS lesions are not uniform, recent data suggest a more heterogeneous scenario. Pioneering neurohistopathological studies [**17, 22**] revealed four different manifestations (pattern I-IV) of MS plaques. Based on their dominant cellular traits,

demyelination was suggested to co-occur on a background of two distinct processes: 1) macrophage and antibody mediated demyelinating processes (Pattern I-II lesions) or 2) primary OL injury-induced demyelinating events (Pattern III-IV lesions), respectively. While pattern I lesions showed typical perivenous distribution, sharply demarcated edges and had active demyelination with inflammatory infiltrates mainly composed of T cells and macrophages; pattern II lesions also showed pronounced immunoglobulin G (IgG) reactivity and complement deposition within active lesions. In contrast, pattern III lesions showed the striking cellular traits of profound OL apoptosis (nuclear condensation and fragmentation) at lesion edges but not in the centre. In particular, lesions appeared with ill-defined borders, did not follow a perivenous extension and thin layer of myelin was often still present around inflamed vessels. Additionally, OL death was associated with the substantial loss of myelin associated glycoprotein (MAG) compared to other myelin proteins in the injured tissue. Pattern IV lesions shared histological similarities with the characteristics of Pattern I lesions, involving plaque geography, the synchronous fashion of myelin protein loss as well as the cell types comprising inflammatory infiltrates with no complement or Ig deposition. Notably, there was strong resemblance of Pattern III lesions with the extensive loss of OLs in active and inactive plaques as well as the presence of DNA fragmentation in OLs in the periplaque WM; suggestive of a functional disturbance of OLs (dystrophy/apoptosis). The broad spectrum of MS histopathology challenged the classical immunological concept and raised the question whether a dysregulated inflammatory process or primary neurodegeneration/ OL death may be the initial event starting new lesions. In subsequent studies by Barnett et. al, this novel initial neurodegenerative concept was further investigated [**18**]. Immunopathological examinations carried out on new symptomatic lesions showed the early presence of apoptotic OLs accompanied by a very early activation of microglia, while infiltration of T cells, macrophages and astrocytes were absent in the apoptotic zone. Based on their findings they proposed that there is a temporal evolution of pathological processes in MS lesion formation [**18, 23**], in which OL apoptosis induced structural and molecular changes in myelin sheath triggers the activation of local microglia and subsequently provokes a systemic immune response. Thus, they profoundly shifted the focus to apoptotic OL death that may not be only a dominant feature in type III MS lesion [**17**], but rather represent an initial point in lesions of MS exacerbations [**18**].

Regardless of these observations there has been no MS specific trigger identified yet to induce either a robust autoimmune response or a progressive cytodegenerative process. However, it is undisputable that both processes play a key role in the temporal and inter-individual heterogeneity of MS lesion pathology and in the broad spectrum of disease courses. Therefore,

details of molecular neuropathology of tissue injury are of the main sources of the management of MS therapy.

1.1.1 Cuprizone-induced experimental demyelination model

The cuprizone-induced experimental demyelination is one of the most commonly applied model to study the mechanism of demyelination and remyelination with a relatively intact blood-brain barrier (BBB) and no signs of an adaptive immune response [**24, 25**]. Cuprizone [Bis(cyclohexanone)oxaldihydrazone] is a copper-chelating reagent originally discovered in the late 1960's [**26, 27**]. Pioneering studies have revealed that systemic administration of the compound- by feeding mice with 0,2% cuprizone mixed into powdered rodent chow- induces a highly reproducible demyelinating brain pathology in a well-defined spatio-temporal fashion [**28**]. The main pathological event in cuprizone –induced lesions is the selective apoptotic death of mature oligodendrocytes (OL) in particular brain regions. Notably, OL death is preceded by the appearance of enlarged mitochondria in the affected cells [**27, 29**]. Although the exact mechanism underlying apoptosis of OLs is still unclear, recent studies proposed that it is partly mediated through possible mechanisms involving mitochondria-linked metabolic failure and increased oxidative stress due to the copper-chelating properties of the agent [**30-32**]. Death of OLs is followed by massive demyelination in well-defined white matter (WM) tracts such as the corpus callosum, anterior commissure or the superior cerebellar peduncules. Beyond these predilection sites, other areas (i.e.: hippocampal formation, cerebellum, caudate putamen, ventral part of the caudate nucleus) or even distinct gray matter (GM) regions (i.e.: striatal complex) and the cortex are also affected, but largely spares the spinal cord [**31, 33-38**]. Besides the selective vulnerability of mature OLs, the histopathology of lesions is also associated with a profound astrocytic activation and microglia/macrophage invasion. Until now, the exact role of these cell types still remained controversial, both activated astrocytes and microglia have been proven to exert beneficial as well as detrimental functions under different experimental conditions [**39-42**]. However, the dynamics and density of astrogliosis as well as microgliosis/macrophage invasion appear with a temporal correlation of changes in myelin protein gene expression (MBP, PLP, MAG), indicating their key role influencing the damaged microenvironment [**43, 44**]. Another important feature of this model is that the length of cuprizone exposure also alters remyelination capacity. After 6 weeks of cuprizone treatment the peak demyelination is reached within the GM and WM, a process termed acute demyelination. When cuprizone challenge is ceased after 6 weeks and animals return to normal chow, acute demyelination is shortly followed by almost complete spontaneous remyelination

driven by the repopulating and maturating oligodendrocyte progenitor cells (OPC). In contrast, prolonged cuprizone administration for 12 weeks or longer induces chronic demyelination, in which spontaneous remyelination is impaired or even fails to occur [**33**].

The precise mechanism or trigger underlying the development of demyelinating lesion in MS is still unknown. It is well-established that most MS lesions occur on an autoimmune inflammatory background. However, previous pathological findings highlighted a possible alternative process, in which lesions exhibiting primary OL death would rather indicate a primary neurodegenerative event as initial steps in lesion genesis [**17, 18**]. Regarding the latter, the histopathology of cuprizone lesions represent significant similarities of the human type III MS lesions [**45, 46**]. Cuprizone-induced primary OL death is preceded by very early downregulation of MAG compared to other OL/myelin specific mRNA levels. As detectable OL apoptosis occurs it leads to demyelination and concurrent activation of astrocytes as well as microglia/macrophages to clean myelin debris and axonal damage [**43, 44, 47**]. However, one notable difference is that it lacks the significant lymphocyte infiltration which is present in human lesions [**48**]. Overall, the cuprizone-induced experimental demyelination model is indispensable for examining the initial neurodegenerative cascades mimicking MS lesion genesis and remyelination. Additionally, the use of this model also harbours numerous possibilities to investigate cellular and molecular traits of non-immune mediated primary demyelination and remyelination of MS ultimately paving the way for novel treatment strategies.

1.2 Overview of primary cilia and their function in the brain

Primary cilia are solitaire, non-motile organelles on the surface of most mammalian cell types. Although primary cilia are similar to motile cilia/ flagella, they do not propagate fluid flow or movement. Instead, they are generally considered as unique sensory and signalling platforms that mediate signal transduction towards the cells which subsequently modifies cellular processes in response to different environmental cues. Importantly, the primary ciliary membrane have been demonstrated to harbour cell type/organ specific receptors as well as signalling molecules that play a critical role in the coordination of numerous developmental and physiological signalling pathways in the mammalian organ system [**49-53**].

The functional importance of primary cilia-mediated signalling in organogenesis and postnatal physiology has become one major focus of interest in the last decade, particularly in the CNS. Studies revealed that proper primary cilia transduced signalling plays a key role in brain tissue

patterning during embryonic stages as well as regulating migration, differentiation and maintenance of stem/progenitor niche [**54-60**]. Additionally, cellular loss of primary cilia or impaired function of ciliary proteins results in abnormal signal transduction towards the cells, which is thought to underlie a wide range of human genetic disorders, collectively termed ciliopathies [**61-64**]. Some human ciliopathies such as Bardet-Biedl Syndrome [**65-67**], Joubert Syndrome [**68-70**], Alström Syndrome [**71-73**], or Meckel-Grüber Syndrome [**74, 75**] comprise severe central nervous system (CNS) involvement including cognitive deficits, mental retardation, and brain malformations.

Although the importance of dysfunctional primary cilia has become undisputable in developmental processes, their precise physiological roles in the adult brain are still vaguely known. In the CNS, primary cilia on neurons are known to be enriched for specific G protein-coupled receptors (GPCRs) including somatostatin 3 receptor (Sstr3) [**76**], melanin-concentrating hormone receptor subtype 1 (Mch1r) [**77, 78**], serotonin receptor 6 ($5HT_6$) [**79, 80**], dopamine receptor 1 (D1r) [**81**], kisspeptin receptor 1 (Kiss1r) [**82**], neuropeptide Y 2 and 5 receptor (NPY2r and NPY5r) [**83**], as well as downstream signalling molecules such as type 3 adenylyl cyclase (AC3) [**84**]. Notably, ciliary expression of these signalling proteins are known to be restricted to different subsets of neurons in the brain. Primary cilia have been implicated in the regulation of the hypothalamus, the main controlling centre of feeding behaviour. Studies have highlighted that specific molecules concentrated in cilia of hypothalamic neurons—such as Mch1r, AC3 and Bardet-Biedl Syndrome proteins (BBS)—contribute to the complex signalling pathways coordinating appetite [**77, 78, 85-87**]; and genetic loss of ciliary structure or certain proteins have been shown to profoundly compromise signalling cascades that leads to hyperphagia-induced obesity in mice under different experimental conditions [**88, 89**]. Additionally, disruption of neuronal primary cilia (NPC) in the hippocampus have been proven to have a significant influence on neuronal connections and adversely affect learning, memory, as well as novel object recognition in mice [**85, 90**]. Possible functions of primary cilia are also reflected by changes in ciliary morphology and length. Adaptation of cilium length—such as elongation—has been proposed to fine-tune the signalling activity of the organelle in response to changes in extracellular environment [**91, 92**]. Moreover, abnormal signalling indicated by either altered morphology or the loss of ability to adjust might be pathological hallmarks of NPC related diseases. Similarly to GPCRs, ADP ribosylation factor-like protein 13B (Arl13b) also localizes to primary cilia and plays a direct role in the initiation, differentiation, and elongation of the organelle [**93-96**]. Cells lacking functional Arl13b exhibit significantly shortened and structurally altered cilia, whereas overexpression of

Arl13b increases ciliary length on the cells [**95-97**]. In line with this, studies have also reported that pharmacological activation, inhibition or genetic absence of other ciliary signalling components can also influence cilium length and morphology under different experimental conditions [**98-100**]. Taken all data together, it is undeniable that primary cilia can dynamically adapt to environmental cues by altering its molecular components and morphology. Besides their well-established role in CNS development, neurogenesis and homeostasis [**54, 56, 59**], recent experimental data drawn from rodent models and *in vitro* cell lines suggest that impaired functions of primary cilia (reflected by altered morphology, signalling protein localisation or its absence) might contribute to the pathogenesis of certain types of CNS cancers [**101-105**] or other neurological disorders [**106-119**].

Despite all of these observations, our knowledge about the precise, physiological roles of primary cilia in the adult CNS are scarce and still not fully understood. Moreover, currently available information about primary cilia in human tissues are mostly limited to human *in vitro* cell lines due to the species and organ specific signalling complexity of the organelle. Nonetheless, detailed neuropathological and cellular characterization of primary ciliary traits in the mature rodent CNS provide an essential tool to investigate their pivotal role in the brain, which might also propagate a better understanding of their clinical relevance and promote novel identification methods for the analysis of post-mortem human tissues in the future.

2. SUMMARY OF THE STUDIES

2.1 Study 1: TRPA1 deficiency is protective in cuprizone-induced demyelination- A new target against oligodendrocyte apoptosis

2.1.1 Background and aim of the study

MS is a chronic, demyelinating and degenerative disease of the CNS. The formation of demyelinating lesions is pathologically a heterogeneous scenario [**17**] and recent data indicate a primary degenerative process in the initial disease phase [**18, 23**]. Primary apoptosis of OLs has been suggested to be an earliest point in lesion evolution, which results in the exposure of naturally hidden CNS antigens that trigger a subsequent autoimmune inflammatory process. Additionally, OL loss becomes even more prominent by the progression of the disease course (SPMS, PPMS) [**120**]. Currently available immunomodulatory therapies are effective to modify the inflammatory nature of the disease, but have mild efficacy on long term clinical course. Thus, there is an increasing need of new pharmacological targets to extend the management of MS therapy for progressive stages. Cuprizone-induced experimental demyelination model is frequently used to recapitulate primary OL pathology and the cellular environment (astrocyte and microglia/macrophage activation) of pattern III-IV MS lesion [**31, 46**].

Transient Receptor Potential Ankyrin 1 (TRPA1) receptor is a member of the TRP channel superfamily [**121**]. It is a nonselective cation channel with relatively high Ca^{2+} permeability. TRPA1 can be activated by numerous endogeneous and exogeneous molecules that are released during inflammation, oxidative stress or tissue injury [**122-130**]. It has a well-established role in pain, mechanical stimuli and cold sensation [**126, 131, 132**]. Due to it widespread expression in different tissues (neural as well as non-neural cells) [**133, 134**], TRPA1 has also been suggested to play an important role in different inflammatory human diseases [**135-139**]. Additionally, activation of this receptor has been also implicated in the regulation of astrocyte functions in the CNS [**140**].

The goal of the study was to investigate 1) whether TRPA1 is expressed in the mouse CNS, 2) whether TRPA1 expression is restricted to specific cell types, 3) whether the genetic deficiency of TRPA1 has a functional role in cuprizone-induced demyelination. To assess these possibilities we applied the cuprizone model on wild-type (WT) and TRPA1 deficient mice bred on a common C57BL/6 background. Our set of experiments included a detailed comparative histopathological analysis to characterise the cellular traits of cuprizone-induced demyelinating lesions and conducted a different set of *in vitro* molecular biological experiments to determine the expression of genes or proteins linked to OL apoptosis and survival.

2.1.2 Results

2.1.2.1 TRPA1 expression in the CNS

To asses the possible role of TRPA1 in the cuprizone-induced experimental demyelination model first we investigated its expression in the mouse CNS. Studies by RT-qPCR revealed that TRPA1 is expressed in numerous brain regions including the corpus callosum (CC) in wilde-type (WT) mice. Additionally, immunohistochemical (IHC) double fluorescence labelling showed that TRPA1 overlaps with GFAP positive astrocytes both in cuprizone naïve and treated mice.

2.1.2.2 Cuprizone-induced demyelination is attenuated in TRPA1 deficient mice

Further IHC studies demonstrated that cuprizone-induced severe demyelination in both cuprizone treated WT and TRPA1 KO mice. Semiquantitative scoring of myelin damage (LFB/CV sections) revealed that cuprizone significantly impaired the myelin score in WT (myelination score: 2,6) and TRPA1 KO (myelination score: 1,7) mice to compared with the control groups (myelination score: 0,2). Importantly, demyelination was significantly less severe in cuprizone treated KO mice compared to WT mice (***P<0.001). Further comparative analysis of markers for mature myelin synthetizing OLs (MBP and MAG) showed that MBP loss was also diminished in cuprizone treated TRPA1 KO mice. Additionally, immunblotting analysis of MAG expression levels were essentially consistent with the differences observed in the above mentioned IHC results.

2.1.2.3 Cuprizone-induced mature OL loss, gliosis and macrophage reactions are reduced in TRPA1 KO mice

On a cellular level, cuprizone treatment profoundly reduced the number of mature OLs in the CC of both WT and KO mice compared to untreated groups (***P<0.001), however, the extent of mature OL loss was significantly less pronounced than in cuprizone-treated WT mice (***P<0.001). The selective death of mature OLs is accompanied by a prominent astrocyte and microglia/macrophage activation in the curizone-induced demyelinating lesions [**32**]. Cuprizone exposure triggered a robust activation of microglia/macrophages and astrocytes in WT mice as indicated by Iba-1 and GFAP IHC. Notably, we found these reactions to be less prominent in toxin exposed KO mice.

2.1.2.4 Number of premature oligodendrocytes is increased in cuprizone-treated WT, but not in TRPA1 KO mice

Next, we investigated whether the diminished loss of mature OLs in TRPA1 KO mice is due to an enhanced remyelination achieved by newly formed, proliferating OPCs or is a result of a possible increased survival of mature OLs. Double immunolabeling technique confirmed the same differences observed in the number of Olig2/APC double positive cells (markers for mature OLs) in the CC of cuprizone-fed WT and KO mice. Importantly, the number of cells immunoreactive for Olig2 only (considered as early OPC) [**141**] was also increased in cuprizone treated WT mice compared to KO mice, while no significant differences were found between the untreated groups. Moreover, gene expression analysis showed that cuprizone exposure triggered a significant *NG2* mRNA elevation (marker for OPC activation to counteract the depletion of mature OLs) in WT (**P < 0.01), but not in TRPA1 KO mice.

2.1.2.5 Expression of *Bak, IGF-1, FGF-2* and *PDGFRα* mRNA in the CC

To determine the mechanism of cuprizone-induced OL loss in the CC we performed a set of growth factor- specific qPCR analyses known to be involved in myelination processes. *IGF-1* mRNA analysis revealed a significant upregulation of *IGF-1* in both cuprizone-treated WT (***P < 0.001) and KO (***P < 0.001) animals. However, cuprizone triggered *IGF-1* mRNA elevation was nearly half of the level observed in WT compared to KO mice (***P < 0.001). *PDGFRα* mRNA expression was not affected by cuprizone treatment in any of the experimental groups. *FGF-2* mRNA expression was increased in both naïve and cuprizone-treated TRPA1 KO animals, but a statistically significant upregulation was only observed in cuprizone-fed WT animals (**P < 0.01). Significant upregulation of *Bak* mRNA was only detected in treated WT groups (***P < 0.001).

2.1.2.6 TRPA1 deficiency attenuates the apoptosis of mature OLs by primarily suppressing the activation of p38-MAPK and c-Jun as well as by enhancing ERK1/2 pathways

Activation of TRPA1 has been linked to MAPK signal transduction cascades [**142-144**]. To further clarify the role of TRPA1 in the cuprizone model we investigated whether the absence of TRPA1 could influence those specific signalling pathways that are linked to OL death in the cuprizone model. Immunoblotting analyses revealed that cuprizone feeding significantly induced the phosphorylation of JNK, ERK1/2 (**P<0.01 and *P<0.05), and c-Jun (*P<0.05) in WT animals. A similar tendency was seen for p38, but it did not reach statistical significance

in the latter group. Absence of TRPA1 did not enhance the cuprizone-induced phosphorylation of p38-MAPK and ERK1/2 to reach a significant level. However, TRPA1 deletion decreased p38, c-Jun phosphorylation (*P<0.05 and *P<0.05) and increased ERK1/2 phosphorylation (**P<0.01) in cuprizone naïve KO mice. Basic level of phosphorylated JNK was increased in untreated TRPA1 KO mice (**P<0.01), and cuprizone treatment did not influence its expression in this group. Additionally, cuprizone treatment did not affect the phosphorylation of MAPK downstream target c-Jun in the KO group.

2.2 Study2: Quantitative comparison of primary cilia marker expression and length in the mouse brain

2.2.1 Background and aim of the study

Primary cilia are small, special cellular organelles that provide important sensory and signalling functions during the development of mammalian organs as well as in the coordination of postnatal cellular processes [**49-53, 87, 145**]. Dysfunction of primary cilia are thought to be the main cause of ciliopathies, a group of pleiotropic human genetic disorders characterized by overlapping developmental defects and prominent neurodevelopmental features [**61-64**]. Besides congenital anomalies in the brain, disrupted cilia-linked signalling pathways have been implicated in the regulation of numerous neuronal functions (e.g.: central regulation of appetite, learning, memory and cognitive functions, olfaction or regulation of gonadal hormone axis) [**82, 85, 88-90, 146-148**], cell-cycle control during perinatal and postnatal life [**53, 145**] and different types of cancers such as glioma and medulloblastoma [**101-105**]. Importantly, studies of recent years have highlighted that different functions of primary cilia are reflected by their diverse morphology and unique signalling components localized in the ciliary membrane. In the CNS, primary cilia on neurons (neuronal primary cilia/NPC) are known to be enriched for a specific subset of G protein-coupled receptors (GPCRs) and signalling proteins- such as somatostatin 3 receptor (Sstr3) [**76**], downstream signalling molecule type 3 adenylyl cyclase (AC3) [**84**] and ADP ribosylation factor-like protein 13B (Arl13b) [**93-96**]- that selectively localize to NPC. Despite of these observations, our knowledge about the precise roles of these organelles in the adult brain are still elusive and are just beginning to be understood.

To further investigate the relationship between primary cilia and brain functions, we conducted a comparative histopathological analysis of primary ciliary traits within the CNS. More specifically, we aimed to characterise 1) the regional distribution, 2) the length and 3) cellular localization of AC3, Sstr3 and Arl13b expressing primary cilia in the adult mouse brain

under physiological conditions. Quantitative comparison of cilia traits were investigated by immunohistochemical studies in 19 different brain regions.

2.2.2 Results

2.2.2.1 Characterization of AC3, Sstr3, and Arl13b expressing primary cilia of CNS cell types

To assess the distribution of ciliary markers in the mouse brain, we first analysed the localization of AC3, Sstr3, and Arl13b positive primary cilia of different CNS cell types. Brain sections were co-immunolabelled with antibodies to the three ciliary markers and with NeuN for neurons, GFAP for astrocytes, APC for mature oligodendrocytes or Iba-1 for microglia/macrophages. In line with previous reports [**76, 84**], the majority of AC3 positive and all Sstr3-positive primary cilia were detected on the surface of NeuN-labelled neurons. While Sstr3-positive cilia were not found on the other investigated cell types, co-labelling also revealed the rare presence of AC3-positive cilia on GFAP-stained astrocytes [**84, 94**]. Occurrence of Arl13b immunopositive cilia was strongly associated with GFAP positive astrocytes. Although we also observed Arl13b positive primary cilia on neurons [**94**], the signal intensity of these cilia were faint and less apparent compared to the other two markers. Neither AC3 nor Arl13b immunoreactive cilia were observed on Iba-1-labelled microglia/macrophages or APC positive mature oligodendrocytes.

2.2.2.2 Comparative distribution of neuronal and astrocytic primary cilia in the mouse brain

It has been previously reported that expression of certain ciliary markers are restricted to different regions of the brain [**76, 84**]. To investigate whether there is a regional preference of primary cilia marker expression, we counted and compared the number of $AC3^+/NeuN^+$ and $Sstr3^+/NeuN^+$ neuronal primary cilia ($AC3^+$NPC and $Sstr3^+$NPC) as well as the $Arl13b^+/GFAP^+$ positive astrocytic primary cilia ($Arl13b^+$AsPC) of distinct areas of the brain.

We found that AC3, Sstr3, and Arl13b positive primary cilia were evenly distributed in the cingular, sensory, motor, and piriform cortices omitting the most superficial layer. Both AC3 and Sstr3 showed the highest density in the piriform cortex where 53.73 % and 29.68% of the cells had AC3- and Sstr3- positive cilia, respectively. The number of $Arl13b^+$AsPC was significantly less in all cortical regions (11.56–16.59%). High number and a higher density of $AC3^+$NPC were found in the olfactory tubercule (91.8%), accumbens nucleus (85.36%), and caudo/putamen (81.3%), whereas a low number of $Arl13b^+$AsPC (8.2–18.69%) and no Sstr3

immunoreactive neuronal cilia were detected in these regions. In contrast, Sstr3$^+$NPC were observed in the claustrum and endopiriform nucleus (21.51% and 29.21%); however, their number was significantly lower compared to AC3$^+$NPC (61.49 and 63.66%).

In the hippocampus, both AC3$^+$NPC and Sstr3$^+$NPC were detected in all subregions of the stratum pyramidale. The number and density of Sstr3 followed a decreasing tendency, namely CA3 > CA2 > CA1, respectively. Notably, we observed a CA2 intersecting area where Sstr3 immunoreactivity only appeared in a punctate form surrounding the cell nuclei. The overall expression pattern of Sstr3 was found to be inversely proportionate to the number of AC3$^+$NPC, including the dentate gyrus. In contrast to the neuronal ciliary markers, Arl13b$^+$AsPC showed an even distribution in the stratum oriens and radiatum bordering the pyramidal layer. Importantly, the density of Arl13b$^+$AsPC was the highest in the stratum lacunosum-moleculare and polymorph layer in the hippocampal formation.

In subnuclei of the amygdala, both AC3$^+$NPC and Sstr3$^+$NPC showed a higher density compared to Arl13b$^+$AsPC, however, the number of AC3$^+$NPC was significantly higher (61.67%) in contrast to Sstr3$^+$NPC (19.2%) or Arl13b$^+$AsPC (19.13%).

The distribution and pattern of primary cilia also varied in the hypothalamic nuclei. Both AC3$^+$NPC and Arl13b$^+$AsPC were detected in all major subnuclei including the paraventricular (PVN), dorsomedial (DM), ventromedial (VM), arcuate (ARC), and suprachiasmatic (SCN) nucleus. The density and number of AC3$^+$NPC and Arl13b$^+$AsPC was the highest in the SCN (85.08%) and PVN (30.93%) regions, respectively. In contrast, ciliary expression of Sstr3 was limited to the VM in which 32.04% of the neurons had Sstr3-positive primary cilia. Although we did not find Sstr3$^+$NPC in the rest of the hypothalamic regions, we also observed a punctate Sstr3 immunoreactivity around cell nuclei similar to the hippocampal CA1-CA2 intersecting pattern.

2.2.2.3 Regional distribution of primary cilia is associated with alterations of their length

Recent studies have demonstrated that ciliary signalling proteins can modulate primary cilia functions by dynamically regulating their length [**100, 119**]. To examine possible regional functions of primary cilia in the CNS, we measured and compared the length of neuronal and astrocytic primary cilia in the mouse brain. We found that the average length of AC3$^+$NPC or Sstr3$^+$NPC was variable in the four investigated cortical regions within the range from 4.88 and 5.51 μm (cingular cortex) to 5.55 and 5.18 μm (piriform cortex), respectively. Besides NPC, the average length of Arl13b$^+$AsPC varied between 2.95 and 3.33 μm. While Arl13b$^+$AsPC

were significantly shorter compared to both types of NPC (***P < 0.001 and/or ****P ≤ 0.001), comparison of AC3 and Sstr3$^+$NPC length did not reach a statistically significant value in either cortical region.

The average length of AC3$^+$NPC were measured essentially longer in the areas of the olfactory tubercule (10.68 μm), accumbens nucleus (9.84 μm) and caudo/putamen (9.61 μm) compared to the length of AC3$^+$NPC measured in the cortices. Additionally, the average length of both AC3 and Sstr3$^+$NPC were similarly short in the claustrum (5.41 and 4.92 μm) and endopiriform nucleus (6.16 and 5.14 μm) as in the cortical areas. Notably, AC3$^+$NPC were significantly longer compared to Sstr3$^+$NPC in the endopiriform region (***P < 0.001). Moreover, the average length of Arl13b$^+$AsPC ranged between 3.37–4.2 μm and was also significantly shorter compared to AC3$^+$NPC and/or Sstr3$^+$NPC in these areas.

In the hippocampal CA regions, the average lengths of AC3$^+$NPC (5.0–5.91 μm) and Sstr3$^+$NPC (3.79–5.46 μm) showed a similar tendency to the cortices. Importantly, AC3$^+$NPC (5.82 μm) were significantly longer compared to Sstr3$^+$NPC (3.79 μm) in the CA1 region (****P ≤ 0.001). In addition, no significant differences were found comparing the average length of the AC3 and Sstr3$^+$NPC in the dentate gyrus (3.2 and 2.78 μm) and subnuclei of the amygdala (6.73 and 6.48 μm). The average length of Arl13b$^+$AsPC in the amygdala and hippocampus were identical to the cortical values (2.8–3.2 μm) and were significantly shorter in all of these regions but the area of the dentate gyrus.

AC3$^+$NPC were longer in the hypothalamus and its nuclei. Their average length varied between 8.0 μm (PVN) and 10.48 μm (VM). Sstr3- expressing NPC were also detected longer in the VM (8.18 μm); however, these primary cilia appeared to be significantly shorter compared to AC3$^+$NPC in this region (**P < 0.01). Repeatedly, Arl13b$^+$AsPC were found significantly shorter (3.6–4.09 μm) in all nuclei of the hypothalamus (****P ≤ 0.001).

3. DISCUSSION, CONCLUSION AND FUTURE PERSPECTIVES OF THE STUDIES

TRPA1 deficiency is protective in cuprizone-induced demyelination— A new target against oligodendrocyte apoptosis

Multiple Sclerosis (MS) is a chronic inflammatory, demyelinating and degenerative disease of the central nervous system (CNS). Currently used immunomodulatory therapies are only able to counteract the inflammatory nature of the disease, however, we do not have strong evidence that these drugs restrain the long-term progression of clinical disability. Thus, adequate management of MS therapy requires new pharmacological targets to inhibit demyelination or promote CNS repair. Transient Receptor Potential Ankyrin 1 (TRPA1) receptor is a non-selective cation channel with relatively high Ca^{2+} permeability. Activation of TRPA1 is linked to well-known environmental substances that produce distinct somatosensory modalities (e.g.: itch and pain) as well as protective responses within different organ systems. However, its pathophysiological role in the CNS has not been elucidated yet. In the present study, we investigated the distribution and possible role of TRPA1 in the cuprizone-induced experimental demyelination model. This toxin-induced model is characterised by primary oligodendrocyte (OL) apoptosis and subsequent demyelination that allows the cellular dissection of the non-immune aspects of MS.

First, we found that TRPA1 is expressed in several distinct regions of the mouse brain and its expression is restricted to astrocytes under physiological conditions. Next, we aimed to assess whether TRPA1 receptor might modulate this toxin-induced demyelination. Comparative histopathological analysis revealed that genetic deficiency of TRPA1 significantly attenuates demyelination and glial reactions (astrocyte and microglia/macrophage) in cuprizone exposed animals. Additionally, we demonstrated that absence of TRPA1 also reduces the selective loss of mature OLs. It is well-known that complex course of toxic demyelination is also accompanied by a robust endogeneous form of reparatory process, in which mature OL loss and tissue damage can be partially or even fully restored by newly recruited and proliferating OPCs to achieve remyelination of lesions [**149-151**]. The evaluation whether the diminished OL death observed in TRPA1 KO mice is due to a reduced vulnerability to cuprizone toxicity or a result of an enhanced endogeneous remyelination process was resolved by a set of IHC and qPCR experiments. We found that cuprizone administration did not increase the number of OPCs (only Olig2 positive cells and *NG2* mRNA expression) significantly in KO mice,

supporting the notion that TRPA1 deficiency reduces apoptotic cell death, but does not trigger a facilitated remyelination. Importantly, further IHC studies highlighted that the distribution pattern of the less prominent cellular reactions coincides with surviving OLs. The notion of diminished glial reactions in KO mice imply a lower degree of tissue injury, which further reinforces the theory that TRPA1 deficiency could be beneficial.

Since we found TRPA1 expression on astrocytes, these histopathological findings prompted us to presume that the absence and presence of the receptor might mediate opposing effects on cellular responses by predominantly affecting astrocytes. Moreover, we speculated a modulatory role in apoptosis- at least partially- by influencing interactions between OLs and astrocytes. TRPA1 activation has been reported to influence physiological functions of astrocytes [**140, 152**]; and growth factors (GFs) released by astrocytes have been demonstrated to influence the fate of cells participating in the myelination processes, such as regulating the proliferation and differentiation of OPCs as well as the survival of mature OLs under different experimental conditions [**39, 43, 149, 153, 154**]. Therefore, another set of qPCR experiments were designed to investigate whether genetic deficiency of TRPA1 might mitigate cuprizone-induced mature OL death by altering the expression of GFs secreted by astrocytes. Upon cuprizone exposure, mRNA levels of *FGF-2* and *PDGFRα* did not show significant changes in KO mice, further indicating that preserved myelin status is not achieved by proliferating and differentiating OPCs into mature OLs. Moreover, we observed higher *IGF-1* mRNA expression in both cuprizone fed WT and KO mice. However, its level was significantly lower compared to WT mice (***$P < 0.001$), which might represent the consequence of a less pronounced activation of astrocytes and diminished apoptotic cell death in the absence of enhanced recruitment of OPCs.

Apoptosis of OLs is also connected to mitochondrial dysfunction during toxin-induced demyelination. Cuprizone toxicity has been demonstrated to lead to impaired functioning of major cell stress and death related mitochondrial mechanisms and is also associated with a reduction of the mitochondrial transmembrane potential [**30, 31, 155**]. The Bcl-2 family-involving both anti-apoptotic as well as pro-apoptotic (such as Bax, Bid and Bak) factors -have been reported to be major determinants of the fate of OLs [**156, 157**]. Notably, we found a significantly elevated level of *Bak* mRNA expression after cuprizone treatment in WT mice (***$P < 0.001$), but there were no significant changes in KO animals. These results show strong consistency with our IHC findings suggesting that TRPA1 deficiency reduces the apoptosis of mature OLs during cuprizone diet.

Based on our findings we hypothesised that stimulation of TRPA1- being a non-selective cation channel involved in Ca^{2+} linked signal transmission [**158-162**]- might contribute to apoptotic cell death by allowing a higher intracellular Ca^{2+} concentration, which could lead to a facilitated activation of apoptotic signalling pathways in mature OLs during cuprizone challenge. TRPA1-coupled downstream signal transduction has been connected to members of the mitogen-activated protein kinase (MAPK) cascades [**142-144**]; and cuprizone-induced apoptosis has been demonstrated to act via (c-Jun N-terminal kinase) JNK and p38-MAPK pathways [**163**]. We speculated that these pathways might be possible intersecting points of cuprizone toxicity and TRPA1 action. Immunoblot analyses revealed that the level of JNK activation was consistently high in both naïve and cuprizone exposed TRPA1 KO mice. Additionally, the basic level of p38 activation was significantly lower (*P< 0.05) and also remained unaffected after cuprizone challenge in KO compared to WT animals, suggesting that the absence of TRPA1 might reduce the activation of p38-MAPK pathway and subsequently the death of OLs. Being a downstream target of JNK and p38-MAPK, activation of transcription factor c-Jun is considered to be a pivotal trigger of apoptosis after various CNS insult [**164, 165**]. In contrast to cuprizone exposed WT mice, the basic level of c-Jun was significantly attenuated in naïve TRPA1 KO animals (*P < 0.05) and its level did not change following cuprizone treatment, indicating the reduced apoptosis of OLs in cuprizone exposed TRPA1 deficient mice. Cuprizone intoxication also resulted in ERK1/2 activation, which was further enhanced in absence of TRPA1. Since ERK1/2 activation has been shown to be important in promoting the survival of OLs [**166-168**], our results suggest a pronounced protective effect against mature OL death may be present TRPA1 KO mice. In summary, our findings strongly suggest that TRPA1 contributes to cuprizone-induced OL death by influencing the CA^{2+} influx into the astrocytes and subsequently facilitating the activation of JNK and p38-MAPK pathways resulting in c-Jun activation in mature OLs.

Quantitative comparison of primary cilia marker expression and length in the mouse brain

Primary cilia are tiny antenna-like cellular appendages that provide important sensory and signalling functions in the mammalian organ systems, particularly in the CNS. Several previous studies have shown that primary cilia play a critical role in the development and maintenance of neural homeostasis of the mammalian brain [**169-171**]. Based on earlier observations [**76, 84**], possible functions of primary cilia can be specified by three major features: (1) the signalling molecules concentrated in the ciliary membrane, (2) the length of the structure, and (3) the cellular and regional localisation of the organelle within the CNS. In particular, primary cilia on neurons (neuronal primary cilia/NPC) are known to be enriched for a specific subset of G protein-coupled receptors (GPCRs) and signalling proteins- such as somatostatin 3 receptor (Sstr3) [**76**], downstream signalling molecule type 3 adenylyl cyclase (AC3) [**84**] and ADP ribosylation factor-like protein 13B (Arl13b) [**93-96**]- that selectively localize to NPC; and have been implicated in the regulation of numerous neuronal functions [**82, 85, 88-90, 146-148**]. Despite of these observations, our knowledge about the precise roles of these organelles in the adult brain are still elusive and are just beginning to be understood.

In the present study, we conducted comparative and quantitative histopathological analyses of AC3, Sstr3 and Arl13b expressing primary cilia with their morphological traits in the physiological adult mouse CNS. First, we aimed to characterise AC3, Sstr3 and Arl13b positive primary cilia expression on different CNS cell types. In accordance with earlier observations [**76, 84**] we found that both AC3 and Sstr3 markers are predominantly expressed on neuronal primary cilia (NPC). Notably, double immunolabelling also revealed faint Arl13b- positive primary cilia on neurons, however, clear and apparent Arl13b expression was strongly associated with primary cilia on astrocytes (astrocytic primary cilia/AsPC). Additionally, only a few AC3 immunoreactive cilia localized to astrocytes and neither of the markers were observed together with mature oligodendrocytes (OL) or microglia/macrophages. It has been reported that the expression and frequency of $Sstr3^{+}$NPC show dynamic changes through stages of CNS development [**172**]; and the expression of AC3 and Arl13b on NPC and AsPC appear in a reciprocal manner in brains of young versus adult mice [**94**]. Thus, our findings further indicate developmentally specified functions of primary cilia of CNS cell types, particularly on matured neurons and subpopulation of astrocytes.

Next, we examined and compared 1) the expression pattern, 2) the length, 3) and the rate of occurrence of NPC and AsPC markers in regulatory centres of the brain. We confirm that AC3 is the most abundant ciliary marker which can be detected throughout the brain, whereas the

distribution of Sstr3 has a more restricted expression profile. Notably, the number and length of these NPC were variable and showed region-specific alternations. In particular, $AC3^+$ NPC were observed to be the densest and longest in the olfactory tubercule (fraction of total 91,8% and average length 10,68 μm), supporting previous studies that implicated their significant role in olfaction [**173-176**]. Additionally, the length of $AC3^+$ NPC was also measured longer in regions of the caudo/putamen (9,61 μm) and accumben nucleus (9,84 μm). Since the protein components of primary cilia are thought to reflect distinct functional traits and we found ciliary morphology alike the olfactory tubercule, it is possible that the proper functioning of these regions may also substantially depend on AC3-linked pathways similar to odorant signalling. However, further experiments are needed to address this possibility.

It has been previously demonstrated that the presence of $Sstr3^+$NPC are scarce in areas where AC3 expressing cilia are prevalent [**84**]. Indeed, we show that the number of $Sstr3^+$NPC is inversely proportionate to $AC3^+$NPC counted in regions, particularly in the cortices and hippocampus. In line with earlier observations [**78, 177, 178**], double immunolabelling experiments revealed two distinct expression profiles in the latter regions, indicating that only distinct population of neuron possess Sstr3- positive cilia. Noteworthy, in the hippocampus Sstr3 immunoreactivity only appeared in a punctate form surrounding the cell nuclei the intersecting area of the CA2 hippocampal pyramidal layer. Since Sstr3 is retained in the cellular compartment while AC3 localizes to the ciliary structure, suggest that the precise functions of NPC may vary within neuronal subpopulations. Sstr3-induced signalling has been connected to adenylyl cyclase activity in the hippocampus [**85**], and genetic ablation of both AC3 and Sstr3 has a major impact on several region related functions such as forms of memory in mice [**85, 90, 146, 179**]. Moreover, dynamic changes of ciliary protein expression and length have been also described in response to pharmacological or agonist treatment [**119, 178**]. Thus, it would be interesting to examine whether these specific neuronal primary cilia in the hippocampus may 1) transport receptors from the cells, 2) alter their morphology and length, 3) or modify neuronal function in the adult brain under different experimental conditions such as stress. However, our results regarding the number and density of $Sstr3^+$NPC detected in the dentate gyrus is in contrast with earlier published data [**172**], in which the density of Sstr3 positive primary cilia was observed to increase in a gradual manner and sustained at higher levels in the adult rat brain. Possible reasons underlying this discrepancy may be due to our different immunolabelling technique applied (antibody and detecting method versus antisera, since similar procedure related differences have been earlier described [**180**]) or our results may reflect age- and/or species-associated alternations between the brains of rodents.

Primary cilia and their dysfunction have also been implicated in the hypothalamic control of appetite and feeding behaviour [**83, 88, 89, 147, 148, 175, 181, 182**]. We found numerous $AC3^+$ NPC in all hypothalamic subnuclei, indicating that AC3 positive cilia are probably involved in the central regulation of appetite. Interestingly, ciliary expression of Sstr3 was restricted to the ventromedial nucleus (VM) and only punctate cellular Sstr3 immunoreactivity appeared in the rest of the hypothalamic nuclei, suggesting that certain hypothalamic NPC might have multiple functional properties beyond the regulation of homeostatic functions. Neuronal primary cilia have been demonstrated to express both Sstr3 and kisspeptin receptor 1 (kiss1r) on different population of hypothalamic neurons that are well-known central effectors driving the neuroendocrine axis such as growth hormone (GH) and gonadotropin-releasing hormone (GnRH) secretion [**82, 183-186**]; and Sstr3 expressing primary cilia on GH secreting cell have been suggested to play an important role in sensing somatostatin ligands in the adenohypophysis of mice [**187**]. Additionally, it has been recently reported that cilia length on hypothalamic neurons are actively regulated according to different metabolic necessities and feeding state [**188**]. Thus, our findings strongly support that $Sstr3^+$NPC might mediate the biological effect of somatostatin on neuroendocrine cells and also contribute to the complex regulation of local hypothalamic neural circuitry. Since the hypothalamus is known to harbour a sophisticated inner network, and we measured both $AC3^+$- and $Sstr3^+$ NPC was generally longer (8,0- 10,48µm) within this region; we speculate that enhanced ciliary length might be indispensable to sense multiple ligands and orchestrate a plethora of different signal transduction pathways for region-specific functions. Additionally, we presume that longer ciliary length may also correlate with the ability to alter ligand binding sites and amplify the efficacy of signal transduction by transporting receptors from the neuronal plasma membrane. However, further studies are needed in the future to elucidate such possibilities. Although the presence of a single primary cilium on the surface of astrocytes have been long accepted and described [**84, 177, 189, 190**], less is known about their precise physiological functions on astrocytes. We confirmed that the majority of Arl13b- positive primary cilia localize to the surface of astrocytes (Arl13b- positive astrocytic primary cilia/Arl13b+AsPC), while AC3 immunoreactivity was scarce, only a few AC3 positive primary cilia were detected on these cell types. Considering their paramount importance in the homeostatic maintenance of the brain [**191, 192**], this preferential expression profile suggest that there might be different populations of astrocytes and Arl13b-signalling may provide an additional mechanism in regulating their region dependent functions. Notably, the number and density of $Arl13^+$AsPC was higher in layers surrounding the hippocampal stratum granulare, a region important in generating new

neurons from adult stem/progenitor cells [**193, 194**]. Primary cilia in the CNS are known to mediate critical developmental signalling pathways such as the Sonic Hedgehog (Shh) pathway [**54, 195, 196**]; and conditional deletion of Arl13b or other ciliary structural proteins disrupt cilium mediated Shh signalling [**95, 97**] as well as hippocampal neurogenesis in the mouse brain [**56, 197**]. Therefore, we presuppose that Arl13b signalling through the non-germinal astrocytes may contribute to a permissive environment to maintain neurogenesis in the dentate gyrus during perinatal and postnatal life. Notably, AsPC were measured significantly shorter compared to NPC within all investigated brain regions, however, in some of the areas—linked average lengths of Arl13b expressing cilia differ from those previously described [**94**]. We assumed the divergence arose from the experimental technique/protocol and antibody applied likewise the disparate expression pattern of $Sstr3^{+}$NPC observed in the hippocampal formation. Arl13b belongs to the Ras GTPase superfamily and primary cilia-mediated Arl13b signalling is required for ciliary microtubule organization, ciliary membrane trafficking pathways, neuronal migration and formation of polarized radial glial scaffold in the developing nervous system [**93, 97, 198-201**]. Signalling through AC3+AsPC have been suggested to provide and additional mechanism affecting synaptic transmission [**84**], thus we speculate speculate a similar regulatory role influencing the biological functions of astrocytes. Nevertheless, the general presence and functional implications of Arl13b on astrocytic cilia is yet to be investigated in future experiments.

Conclusion and future perspectives

TRPA1 deficiency is protective in cuprizone-induced demyelination—A new target against oligodendrocyte apoptosis

Multiple sclerosis (MS) is a chronic, inflammatory, degenerative and progressive disease of the central nervous system (CNS). The exact cause of the disease is still unknown and there is no available cure for it. Currently used immunomodulatory therapies are only able to counteract the inflammatory nature of the disease and these drugs have little efficacy on long term progression of clinical disabilities. Thus, adequate management of MS therapy requires new pharmacological targets to inhibit demyelination or promote CNS repair. In the present study we investigated the expression and possible role of TRPA1 in the cuprizone-induced experimental demyelination model. We show that TRPA1 is expressed on astrocytes in the mouse CNS under physiological conditions. Moreover, we demonstrate that the genetic deficiency of TRPA1 significantly attenuates the demyelination in cuprizone exposed animals by reducing the apoptosis of mature OLs Based on our data, we conclude that opening of these multisteric ion channels triggers the elevation of intracellular Ca^{2+} concentration. Activation of TRPA1 receptor- either indirectly by modulating astrocyte function and consequently astrocyte-OL crosstalk; or directly by acting on OLs—may contribute to OL death by supporting the pro-apoptotic p38-MAPK pathway resulting in c-Jun activation and consequent OL apoptosis. However, our data rather support the indirect effect of TRPA1 activation on OL death. We propose that inhibition of TRPA1 might successfully diminish the non-immune mediated degeneration in MS and could be promising drug candidates to limit CNS damage in demyelinating diseases.

Quantitative comparison of primary cilia marker expression and length in the mouse brain

Primary cilia are tiny antenna-like cellular appendages that provide important sensory and signalling functions in the mammalian organ systems, particularly in the CNS. In recent years, the clinical and biological relevance of these organelles gained increasing attention, which uncovered that primary cilia dysfunction may be the underlying cause of numerous human diseases such as congenital ciliopathies, certain types of CNS tumors or other neurological disorders. Although primary cilia are found to be widely distributed in the brain, the precise role of these organelles are just beginning to be understood. Notably, studies of the last decade have highlighted that functions of neuronal cilia are reflected by the signalling molecules enriched in the ciliary membrane, their morphology, and localization in the CNS. In the present study, we conducted a comparative and quantitative histopathological analysis of the expression pattern, distribution and length of primary cilia expressing AC3, Sstr3 as well as Arl13b in the adult mouse brain. We show that primary cilia of neurons and astrocytes display a well-characterised ciliary marker expression profile throughout the investigated brain regions. Additionally, quantitative comparison of their length, density and occurrence rate revealed apparent differences among regulatory centres of the CNS, further indicating possible, yet unknown roles of primary cilia in brain functions. Overall, our study provides a comprehensive overview of the cellular organization and morphological traits of primary cilia in regions of the physiological adult mouse brain, which serves an important tool in understanding the role of these organelles in future experiments. Additionally, considering the genetic aetiology of numerous human neurodegenerative diseases, the characterization of dysfunctional primary cilia on post- mortem human brain tissues might provide a different aspect of understanding disease pathogenesis and may open novel therapeutic avenues for clinical management of neurological disorders such as Parkinson’s disease or Multiple Sclerosis.

4. LIST OF PUBLICATIONS

4.1 Articles related to the thesis

Sághy*, É., **Sipos*, É.**, Ács, P., Bölcskei, K., Pohóczky, K., Kemény, A., Sándor, Z., Szőke, É., Sétáló, Gy. Jr., Komoly, S. and Pintér, E. (2016). "TRPA1 deficiency is protective in cuprizone-induced demyelination-A new target against oligodendrocyte apoptosis." *Glia, 64*(12), 2166-2180. doi: 10.1002/glia.23051
*contributed equally

Bölcskei, K., Kriszta, G., Sághy, É., Payrits, M., **Sipos, É.**, Vranesics, A., Berente, Z., Ábrahám, H., Ács, P., Komoly, S. and Pintér, E. (2018). "Behavioural alterations and morphological changes are attenuated by the lack of TRPA1 receptors in the cuprizone-induced demyelination model in mice. " *J Neuroimmunol, 320,* 1-10. doi: 10.1016/j.jneuroim.2018.03.020

Sipos, É., Komoly, S. and Ács, P. (2018). "Quantitative Comparison of Primary Cilia Marker Expression and Length in the Mouse Brain." *J Mol Neurosci, 64*(3), 397-409. doi: 10.1007/s12031-018-1036-z

4.2 Abstracts published in cited journals

Pintér, E., Bölcskei, K., Sághy, É., Payrits, M., Kriszta, G., Vranesics, A., **Sipos, É**., Ács, P., Berente, Z., Ábrahám, H., Komoly, S. (2017) "TRPA1 receptor deficiency substantially diminishes the cuprizone-induced demyelination. " *Journal of Neurochemistry* 142 : 1 pp. 171-172., 2 p.

4.3 Poster presentations

Sipos, É., Komoly, S., Ács, P. "Expression of primary ciliary markers in satiety centers of the brain. " Department of Neurology, Medical School of Pécs, University of Pécs, Hungary. P91, *IBRO Workshop*, Debrecen, Magyarország, 2014

Sághy É., Bölcskei K., Perkecz A., Sándor Z., Kemény Á., Szőke É., Ács P., **Sipos É.,** Gaszner B., Komoly S., Helyes Zs., Pintér Erika. "A Tranziens Receptor Potenciál Ankyrin 1 (TRPA1) receptor szerepe cuprizone-indukált kísérletes demyelinizáció modellben. " A Magyar Élettani Társaság 79. Vándorgyűlése és a Magyar Mikrocirkulációs és Vaszkuláris Biológiai Társaság Konferenciája, Szeged, Magyarország, 2015.

Sághy É., **Sipos É.**, Ács P., Bölcskei K., Pohóczky K., Kemény Á., Sándor Z., Szőke É., Sétáló Gy. Jr., Komoly S., Pintér E. "A TRPA1 receptor szerepe a sclerosis multiplex kísérletes állatmodelljében. " Magyar Farmakológiai, Anatómus, Mikrocirkulációs és Élettani Társaságok Közös Tudományos Konferenciája, Pécs, Magyarország, 2016.

Sághy É., Bölcskei K., Péter Á., Komoly S., **Sipos É.,** Szőke É., Perkecz A., Gaszner B., Kemény Á., Sándor Z., Helyes Zs., Pintér E. "Transient Receptor Potential Ankyrin 1 (TRPA1) receptor has regulatory role in the cuprizone-induced demyelination in mice. " *IBRO Workshop,* Budapest, Magyarország, 2016.

4.4 Oral presentations

Pintér E.**,** Sághy É., Payrits M., Bölcskei K., Perkecz A., Sándor Z., Kemény Á., Szőke É., Ács P., **Sipos É.,** Komoly S., Helyes Zs., Ábrahám I. "A Tranziens Receptor Potencial Ankyrin 1 (TRPA1) Receptor szerepe a neurodegeneratív kórképekben**.** " Magyar Farmakológiai, Anatómus, Mikrocirkulációs és Élettani Társaságok Közös Tudományos Konferenciája**,** Pécs, Magyarország, 2016.

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Sipos É., Ács P., Komoly S. "Elsődleges csillók a központi idegrendszerben."
BAW – Brain Awareness Week/ Agykutatás Hete, Pécsi Tudományegyetem, Általános Orvostudományi Kar, 2017. március 13-19.

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