absence for HC. The strongest ones occurred in: cerebellum (FA: $p < 0.001$), deep gray matter (MD: $p = 0.0086$), cortical gray matter (FA: $p = 0.0036$, MD: $p = 0.0086$), cerebrospinal fluid (FA: $p = 0.0062$, MD: $p = 0.002$).

Conclusion: DTMs for several ROIs differ statistically significantly between HC and MS groups. Moreover, longitudinal follow-ups in the MS group demonstrated significant DTM trends, contrasting with the absence of such trends in HC. Likewise, this is crucial to determine the spatial distribution of systematic errors specific to each MR scanner and DTI acquisition protocol to establish accurate DTMs for both individual patients and mean values for a healthy population. This approach allows for an initial reliable diagnosis based on DTMs.

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Developing novel acid-sensing ion channel inhibitor as a neuroprotective lead for multiple sclerosis

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Introduction: The quest for neuroprotective and remyelinating therapies in multiple sclerosis (MS) persists despite advancements in immunomodulatory drugs[1]. Recently, activation of acid-sensing ion channels (ASICs), particularly ASIC1a, ***has been associated with demyelination and axonal injury in postmortem CNS tissues in experimental autoimmune encephalomyelitis (EAE) mouse models and MS patients*** [2-4].

Objectives/Aims: The current study aimed to identify the therapeutic potential of Hi1a, an exceptionally potent inhibitor of ASIC1a (IC50 500 pM), in reducing focal demyelinated plaques in the EAE-mouse model of MS.

Methods: Female C57BL/6 mice induced with EAE[5] were randomised to receive a once-daily intraperitoneal dose of either Hi1a (50 µg/kg) or PBS using blinded experiment conditions. EAE disease progression and motor deficits were evaluated[6,7] and terminal blood and tissues were processed blinded for immunohistochemistry (IHC) and ELISA to study various neuroinflammatory markers relevant to MS. Hi1a conjugated with Alexafluor 700 (Hi1a-AF700) administered to EAE mice at peak disease was used to locate the major site of action of the test-item.

Results: EAE mice treated with Hi1a (50 µg/kg) showed significant amelioration of EAE disease (i.e motor deficits) compared to the PBS group. Mice treated with Hi1a showed significantly reduced demyelination (MBP staining loss), activation of microglia/macrophages (Iba1), astrocytes (GFAP) and myeloperoxidase (MPO), a marker of chronic inflammation in spinal cord tissues when compared with PBS treatment. ELISA assay for MPO expression in spinal cord lysates from EAE mice showed consistent results with that of IHC. Hi1a-AF700 administered to EAE mice was predominantly expressed in neuronal cell bodies in spinal cord tissues. Furthermore, the uptake of Hi1a-AF700 in CNS tissues from EAE mice was confirmed by imaging with Odyssey® DLx scanner.

Conclusion: Hi1a, predominantly interacting with neuronal cell bodies, significantly reduced demyelinating lesions in EAE mice. Future studies are warranted to elucidate its precise mechanism of neuroprotection.

Disclosure of interest: None

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Variability of the cortical excitability in patients with Relapsing-Remitting Multiple Sclerosis and comorbid primary headache

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Introduction: Primary headache (PH) is a common comorbid condition among patients with multiple sclerosis (MS). It may have an impact on the disease course, prognosis and quality of life by changing the cortical excitability in MS patients. Paired transcranial magnetic stimulation (pTMS) is a novel neurophysiological technique to analyze intracortical inhibition (ICI) and facilitation (ICF) that can be used among relapsing remitting MS (RRMS) patients.

Objectives/Aims: The aim of the study is to assess the difference in the values of short ICI (SICI) and short ICF (SICF) between RRMS patients regarding the presence of comorbid PH.

Methods: 76 patients with RRMS were examined by pTMS, where pulses were delivered using a 90-mm circular stimulator coil and connected to a MagPro R30 stimulator. An electromyography (EMG) device was used to record the signal from the abductor major muscle of the dominant upper limb by surface electrodes of the disc in the tendon. The parameters evaluated were the following: motor threshold (MT) in rest, motor evoked potential amplitude (AMEP), SICI 1 and 2 (recording in 2 and 3 sec respectively), SICF 1 and 2 (recording in 12 and 15 sec respectively). Patients were divided into 2 groups: group 1 - with comorbid PH (n=46); group 2 - without comorbid PH (n=30). Statistically the method of estimating the difference of variations by using variance analysis (sigma value) was used.

Results: Mean values of pTMS were the next: MT - 41.98±8.75%, AMEP - 2.04±1.73 mV; SICI1 - 66.62±49.24%; SICI2 - 92.26±83.84%; SICF - 209.47±203,22%; SICF2 - 278.54±194.42%. For both groups variability of AMEP, SICF1 та SICF2 was statistically significant ($p<0.05$). However, the variability of AMEP and SICF1 was higher and the SICF2 was variably lower among group 2 (AMEP sigma 4,272;6,510; SICF1 sigma - 16,742;45,480; SICF2 sigma - 142,664;214,885) compared to group 1 (AMEP sigma: 1,150;1,950; SICF1 sigma: 90,021;151,657; SICF 2 sigma: 175,333;290,133). This shows the peculiarities of intracortical facilitation in RRMS and comorbid PH when recorded at different time intervals, where the amplitude and onset of SICF showed better conduction indices, but with time the facilitation index decreased.

Conclusion: SICI variability was not statistically different among two assessed groups, however SICF pattern change was found in the group with comorbid PH. It means that in RRMS presence of PH may provoke a tendency to increase cortical excitability in the facilitation phase.

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Evidence for Clostridium perfringens epsilon toxin within active MS lesions: Implications for etiopathogensis

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Introduction: Episodic focal white matter inflammatory demyelination characterizes relapsing forms of MS, but the precipitating factor(s) are unknown. Numerous infectious etiologies have been proposed as triggers, with recent emphasis on EBV. We previously reported (Ma, JCI 2023) that 61% of people with MS harbor epsilon toxin (ETX) producing strains of *Clostridium perfringens* (types B or D) in relatively high abundance within their gut microbiome, whereas 13% of healthy controls harbored type B or D strains and in low abundance. We also showed that ETX can induce inflammatory demyelination in an active immunization model of EAE (ETX-EAE). Combined, these two findings raise the possibility that ETX may trigger inflammatory demyelinating white matter lesions in MS.

Hypothesis: ETX is produced episodically in the MS gut during log-phase growth of *C. perfringens* types B or D, enters the blood, binds to CNS endothelial cells where it alters transcription, induces transcytosis, and disrupts tight junctions. These events allow toxin and autoreactive lymphocytes to enter the CNS leading to inflammatory demyelination in a distribution surrounding a central vein.

Objectives/Aims: To determine if ETX is present in active MS lesions and test if an ETX neutralizing antibody prevents disease in the ETX-EAE model.

Methods: To assess for the presence of ETX, we developed high-affinity anti-ETX rabbit polyclonal antibodies. Sections were stained for ETX, proteolipid protein (PLP) and MHC-II.

To determine if ETX neutralizing antibodies abrogate ETX-EAE, C57BL/6 mice were immunized with myelin and oligodendrocyte protein (MOG) in complete Freund's adjuvant and disease triggered by active ETX IP in the presence or absence of ETX neutralizing antibodies.

Results: To assess for the presence of ETX in active white matter lesions we examined well-curated autopsy tissue from three individuals with clinically definite MS (ages 52-60 years; disease duration 12-30 years). Active white matter lesions, defined by loss of PLP staining with sharp margins and positive MHC-II staining, revealed intense staining for ETX. ETX staining overlapped precisely with demyelination defined by the lack of PLP staining.

Normal appearing white matter adjacent to active lesions, as well as control white matter, did not stain for ETX.

In the ETX/MOG EAE model, neutralizing antibodies to ETX, but not control antibodies, significantly attenuated ETX induction of EAE.

Conclusion: We document, for the first time, ETX immunoreactivity in active white matter lesions corresponding directly to regions of demyelination and MHC-II activation. We show that intravenous neutralizing ETX antibodies markedly attenuate disease in ETX-EAE. Taken together with prior work showing a strong association between ETX producing *C. perfringens* and MS, these findings support a role for ETX in triggering episodic white matter demyelination in MS and provide strong rationale for intervention trials targeting ETX.

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