Volumetric Alterations of Subcortical Brain Structures in Female Migraineurs

Doctoral (PhD) Dissertation

Mingchen He, MD

Supervisors:

Prof. Zoltán Pfund, MD, PhD, DSc

Gábor Perlaki, PhD

Program Director: Prof. Zoltán Pfund, MD, PhD, DSc

Doctoral School Director: Prof. József Janszky, MD, PhD, DSc

University of Pécs, Medical School

Doctoral School of Clinical Neurosciences

Pécs, 2024

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List of Abbreviations

A+	patients with aura
A-	patients without aura
ANCOVA	analysis of covariance
ASL	arterial spin labeling
BOLD	blood oxygenation level dependent
CA	cornu ammonis
CM	chronic migraine
CSD	cortical spreading depression
DG	dentate gyrus
DTI	diffusion tensor imaging
EM	episodic migraine
FD	fractal dimension
FLAIR	fluid-attenuated inversion recovery
fMRI	functional MRI
FOV	field-of-view
HC	healthy controls
HF	high frequency
ICHD	International Classification of Headache Disorders
ICV	intracranial volume
IHS	International Headache Society
IM	inflammatory mediators
L+	patients with white matter lesions
L–	patients without white matter lesions
LF	low frequency
LSD	least significant difference
MRI	magnetic resonance imaging
MwA	migraine patients with aura

MwoA	migraine patients without aura
NRM	nucleus raphe magnus
NCF	nucleus cuneiformis
PAG	periaqueductal gray
PET	positron emission tomography
ROS	reactive oxygen species
rsFC	resting state functional connectivity
RVM	rostral ventromedial medulla
SBM	surface-based morphometry
SCP	superior cerebellar peduncle
SD	spreading depression
SMA	supplementary motor area
SN	salience network
SPSS	statistical package for the social sciences
SpV	spinal trigeminal nucleus
TCC	trigeminal cervical complex
US	United States
VBM	voxel-based morphometry
WHO	World Health Organization
WMLs	white matter lesions
YLDs	years lived with disability
3D MPRAGE	three-dimensional magnetization-prepared rapid gradient-echo

1. Introduction

1.1 Background Knowledge of Migraine

1.1.1 Overview of Migraine

Migraine is a common primary neurological disorder, which is an often life-long challenge that is characterized by recurring attacks (Olesen 2018). The frequency of migraine headaches varies from person to person. Typically, a migraine attack involves head pain that is moderate to severe in intensity, often characterized by recurrent throbbing or pounding sensing. Although they strike frequently one side of the head, they may occur anywhere around the head. In the most severe cases, they are usually associated with photophobia, phonophobia, osmophobia and cutaneous allodynia (Ferrari et al. 2022). Nausea is one of the most common symptoms and it worsens with activity, which often leads to patient disability. Migraine attacks usually last from 4 to 72 hours and most people are free from symptoms between attacks. Migraine headaches can be triggered by allergies, stress, smoking, alcohol, strong smells, bright lights, loud noises, skipping meals, dehydration, irregular sleep, low blood sugar, poor posture and hormonal fluctuations (Islam and Nyholt 2022). There are no specific biological markers to help to make the diagnosis of migraine, therefore, to make a precise diagnosis, we mainly rely on the clinical history, and also need to exclude other types of headache disorder. Physicians may use guideline to make clinical diagnosis, and to decide subsequent treatment.

1.1.2 The Prevalence and Economical Impact of Migraine

The overall prevalence of migraines is about 10%–15% of the general population and is associated with a substantial personal and social burden (Ashina 2020; Burch et al. 2018; Lai and Niddam 2020; Lipton et al. 2001; Rasmussen and Olesen 1992; Schramm et al. 2023). Migraine probably begins early in the childhood, but its prevalence

increases quickly at 10 to 14 years old and continues to increase until approximately 40 years old, after that it gradually decreases, especially among women after menopause (Arruda et al. 2010; Sacco et al. 2012). A study in United States (US) found that the 1-year prevalence of migraine is 6.3% in adolescents (5.0% in boys and 7.7% in girls) (Bigal et al. 2007). Another study conducted by the same group demonstrated that people aged 30 to 39 years have the highest prevalence in migraine, with 7.4% and 24.4% prevalence for men and women respectively. Prevalence is the lowest among people aged 60 years or older (1.6% in men and 5.0% in women) (Lipton et al. 2007).

Epidemiological studies have demonstrated high prevalence and high socio-economic and personal impacts of migraine (Bes et al. 2013). It affects large general population, and it is associated with a substantial personal and social burden (Lipton et al. 2001a; Rasmussen et al. 1991). According to the Global Burden of Disease Study 2016, migraine is one of the ten diseases with the greatest prevalence, and is estimated to affect 1.04 billion (95% uncertainty interval: 1.00 billion to 1.09 billion) patients worldwide (Vos et al. 2017). Meanwhile, migraine has become the second leading cause of disability, and ranked in the top ten causes of years lived with disability (YLDs), a health biometric adopted by the World Health Organization (WHO) to measure the burden of a disease (Murray 1994), in 195 countries and territories (Vos et al. 2017). In accordance with the statement of WHO, 50% to 75% of adults aged 18–65 years in the world have suffered headache, and among them, 30% or more have reported migraine. Comparing to those who do not have migraine, people who suffer migraine have direct costs (increased healthcare spending) and indirect costs (decreased productivity), which increases the relevant economic burden. Because of healthcare and lost productivity issues, the costs associated with migraine are estimated to be as much as $36 billion annually in the US (Bonafede et al. 2018).

1.1.3 Do Women Get More Migraines?

Migraine is more common in women than men due to hormonal differences starting in

puberty (Finocchi and Strada 2014; MacGregor et al. 2011; Vetvik and MacGregor 2017), and the prevalence at its peak among women is more than 25% (Allais et al. 2020). Usually there are two to three times as many female migraineurs as male migraineurs (Charles 2017; Shankar Kikkeri and Nagalli 2020). It is worth noting that the female predominance on migraine was consistent across all races and ethnic groups (Loder et al. 2015). The female to male ratio peaked at 3.25 among those between 18 and 29 years of age, because of hormonal influences (Buse et al. 2013). Nearly 60% of women with migraine showed a relationship between migraine and menses (Pavlović et al. 2015). Women with menstrual-related migraine develop headaches between two days before the onset of menses to the third day of menstrual bleeding (Moy and Gupta 2021). Usually, these attacks are more disabling than non-menstrual ones. Hormone estrogen is believed to contribute to this difference. When estrogen levels rapidly drop before menstruation, women have an increased likelihood to develop migraine headaches (Pavlović 2020). It is not the absolute levels of hormones, but more the degree of fluctuations in hormones that cause the migraine attacks (Avona et al. 2019; Pringsheim and Gooren 2004; Somerville 1972). In contrast, increased levels of estrogen may be protective against migraine (Chai et al. 2014). During pregnancy, as estrogen levels rise and remain elevated throughout the pregnancy, migraine headaches improve for the majority of women, and for some of them, their symptoms completely disappear benefiting from high estrogen levels (Ibrahimi et al. 2017; Welch 1994). After pregnancy, however, the abrupt drop in estrogen levels may trigger migraine again.

Although the association between migraine and sex hormones has been repeatedly demonstrated, the related pathophysiology of this association has not yet been fully understood. In addition, the migraine characteristics of men and women are different. Women report a longer attack duration, increased risk of headache recurrence, more frequent nausea, phonophobia, and photophobia, greater disability, and longer time required for recovery (Finocchi and Strada 2014; MacGregor et al. 2011; Vetvik and MacGregor 2017). Some studies reported, in general, females evidenced lower pain

thresholds and less tolerance to noxious stimulation than men (Berkley 1997; Hoffmann and Tarzian 2001). Furthermore, during their menses, the pain thresholds in females have been reported to be lower, moreover, when women are in the condition of low progesterone and high estradiol, there are no significant difference in pain thresholds between women and men (Stening et al. 2007; Unruh 1996). In addition to sex differences on the frequency of migraine, the severity of pain among women and men have also been investigated. Some studies suggested that women have greater pain severity than men (Barnabe et al. 2012; Fillingim et al. 2003; Keefe et al. 2000).

1.1.4 Clinical Features and Imaging in Different Phases

Migraine is characterized by its periodicity and multiple phases: prodrome, aura, headache, postdrome, and the interictal period (Figure 1). During the interictal phase, which is the interval period between two migraine episodes, patients are usually symptom free (Peng and May 2020).

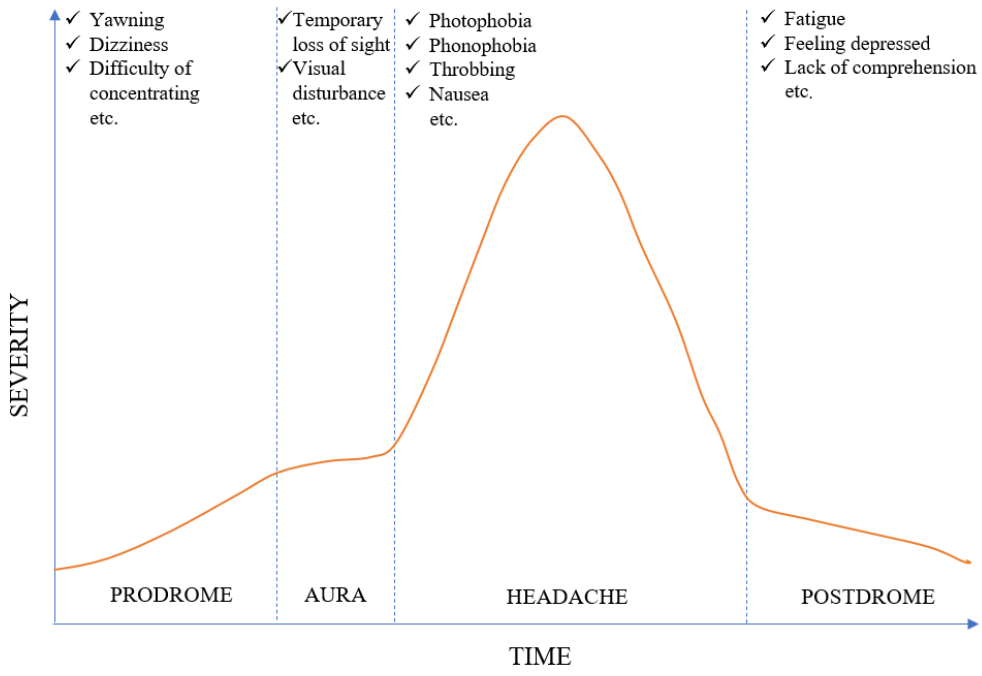

Figure 1. Four phases of migraine attack with corresponding clinical symptoms.

Prodrome is also known as premonitory phase, and this phase can last few hours or may even occur over several days. Prodrome symptoms vary from person to person. The most common symptoms include yawning, dizziness, fatigue, phonophobia, mood changes and food cravings (Schoonman et al. 2006). Neuroimaging has been widely used in migraine studies. Over the recent years, various imaging techniques have been used in the investigation of premonitory phase of migraine. Hypothalamus, midbrain and limbic system have been demonstrated to be involved in central migraine attack initiation by imaging prodrome phase (Karsan and Goadsby 2023). A recent study performed resting-state functional magnetic resonance imaging (fMRI) in individuals with nitroglycerin-triggered migraine, and found increased thalamocortical connectivity and functional uncoupling between the pons and the limbic lobe in the premonitory phase, which indicates alterations in subcortical and brainstem networks in this phase (Karsan et al. 2020). Maniyar et al. (2014) used positron emission tomography (PET) to scan migraineurs without aura who were given nitroglycerin to trigger premonitory symptoms, and found the posterolateral hypothalamus, midbrain tegmental area, periaqueductal gray matter (PAG), dorsal pons and various cortical areas activated in the premonitory phase compared to baseline. Another nitroglycerin-triggered migraine perfusion MRI study found increased blood flow in similar brain regions (Karsan et al. 2018).

The aura phase is the time right before the migraine attack, which may not happen in all migraine patients. Aura is a prominent clinical presentation in the preictal phase for some patients (Borsook et al. 2015). In 1980, Blau (1980) proposed that migraine aura and prodrome should be put in different phases to remove a semantic difficulty. Many others also put aura as a specific phase in migraine (Hansen et al. 2012; Shankar Kikkeri and Nagalli 2020). It happens immediately after the prodromal phase and right before the ictal phase. This phase can last from 5 minutes to 60 minutes (Ferrari et al. 2022). Some have both the aura and the headache at the same time (Hansen et al. 2012). But it is still debated whether aura should be considered as a specific phase, in consideration

of the following factors: 1.) aura only occurs in some of the migraine patients. 2.) For migraineurs with aura, aura does not appear during every migraine attacks. 3.) 3% of female migraine patients and 1% of male migraine patients suffer migraine aura without headache (He et al. 2015; Peng and May 2020). So here we also would like to use the term of “preictal phase”. Regarding the clinical and imaging manifestations of aura, please see section 1.3.

The descriptions of migraine headache vary. Mostly, the pain could disrupt normal activities, and its intensity ranges from merely annoying to disabling. The routine physical activity could worsen the severity of a migraine headache. Typical migraine headaches are unilateral, and then may spread to the other side. A migraine headache usually lasts from 4 to 72 hours if untreated (Ferrari et al. 2022). Photophobia and phonophobia are the most common symptoms associated with migraine, followed by nausea and vomiting (Eigenbrodt et al. 2021). The vascular origin of the headache during migraine attack has been debated for decades. An arterial spin labeling (ASL) perfusion MRI study found regional hyperperfusion during the headache phase (Wolf et al. 2018), whereas, another case study revealed brain hypoperfusion in the ipsilateral brain hemisphere to the symptoms during the headache (Lester et al. 2021). It is worth noting that the subjects in these two studies are too limited, the contradictory conclusions need to be investigated in bigger studies in the future. Schulte et al. (2018) compared migraineurs having headaches during scanning procedure with migraineurs without headaches and healthy controls, and found the activation within the area of the spinal trigeminal nucleus were stronger for migraine patients having headaches during scanning. Another fMRI study demonstrated increased functional coupling between the pons and spinal trigeminal nuclei during migraine headache (Karsan et al. 2020).

Postdrome typically occurs after the headache phase. However, not all individuals with migraine suffer from postdrome; it occurs in approximately 68% of patients, and in 88% of cases it lasts less than 24 hours (Kelman 2006). Fatigue, nausea, and concentration

difficulties are the most common symptoms (Ng-Mak et al. 2011). Imaging studies focusing on postdrome phase of migraine attack are relatively scarce. Bose et al. (2017) used ASL MRI to investigate alterations in cerebral blood flow during the postdrome phase of a migraine attack, and found regional cerebral blood flow reduction in the superior temporal gyrus, middle temporal gyrus, medial frontal gyrus and inferior temporal gyrus during the postdrome phase compared to baseline.

1.2 Mechanisms of Migraine

1.2.1 The Origin

The migraine headache probably originates in the nociceptive sensory fibers, that transmit signals from pial, extracranial and intracranial blood vessels, sinuses and dura mater. (Noseda and Burstein 2013). The trigeminovascular system is a pain-transmission link between the vascular (dural and cortical) and neuronal (brainstem and thalamus) regions (Moskowitz 1992). During a migraine attack, central and peripheral sensitization of trigeminovascular nociceptive pathways may increase (Ashina 2020; Ashina et al. 2021). However, it is still not fully clarified that what generates a migraine attack, although some mechanisms based on activation of the trigeminovascular system are broadly associated (Ashina et al. 2019; Noseda and Burstein 2013).

It is still debated whether migraine is generated from peripheral or central changes. Some earlier research studies had presented a notion that a particular brainstem area may act as the migraine generator (Bahra et al. 2001; Diener and May 1996; Tajti et al. 2001). Later, other researchers suggested that although the brainstem is highly linked to the migraine biology, the functional changes of hypothalamo-brainstem network might be the real driver of migraine attacks (Filippi and Messina 2020; Schulte and May 2016). In animal models, spreading depression can be elicited in the brainstem, but the requirements to trigger it are more extreme than in the cortex (Richter et al. 2008). In spite of the uncertain of exact mechanism of initiation of migraine attack, a

series of activities happen, which includes: initiator (evoked or spontaneous), clinical or subclinical spreading depression, activation of trigeminovascular nociceptors, peripheral and central sensitization of trigeminovascular pathways, brain hypersensitivity and hyperexcitability, and functional and structural changes in the brain (Borsook et al. 2015).

1.2.2 Modulation System of Migraine

The modulation of migraine is mediated by ascending (Figure 2) and descending (Figure 3) pathways. Neurons in the ascending pain pathways receive input from peripheral primary afferent fibers and transmit from the dorsal horn of the spinal cord to a number of supraspinal sites (D'Mello and Dickenson 2008). Descending pain pathways mediate top-down regulation of nociceptive processing, and transmit cortical and limbic influences to the dorsal horn (Heinricher 2016). These regulatory pathways are closely intertwined as part of positive and negative feedback loops (Chen et al. 2017a).

The migraine attack involves activation of the ascending trigeminothalamic pathway. Trigeminal fibres transmit sensory information from intracranial structures, such as the dura matter and intracranial vasculature, synapse on second-order neurons within the trigeminal cervical complex (TCC), which is a key relay center in the transmission of nociceptive information, which has been considered as a standard for modeling headache (Akerman et al. 2013). These neurons give rise to the major trigemino-thalamic ascending pathway, which transmits sensory information to third-order neurons located primarily in the contralateral thalamus, which then process the information to higher cortical areas (Andreou and Edvinsson 2019; Su and Yu 2016).

Descending modulation from brainstem structures have extensive effects on pain processing. PAG, nucleus cuneiformis (NCF), and rostral ventromedial medulla (RVM) can exert both excitatory and inhibitory effects on dorsal horn neurons (Brennan and

Pietrobon 2018). RVM is the primary output structure of a major descending pain-modulating system. In RVM, ON-cells and OFF-cells exert net pronociceptive and antinociceptive effects, respectively (Chen et al. 2017a). An animal study with dural inflammatory mediators (IM)-induced cutaneous allodynia demonstrated ON-cells were potently activated during IM application, whereas, OFF-cells displayed a transient inhibition during and immediately after the IM infusion (Edelmayer et al. 2009). Long-term changes in PAG/NCF output could reduce descending pain inhibition in migraine, and increase the response to algesic stimuli (Brennan and Pietrobon 2018; Ossipov et al. 2010).

An increasing number of evidence indicates that individuals with migraine are mostly non-allodynic during the first few years of their migraine experience, and eventually develop allodynia by following migraine attacks (Burstein et al. 2000; Mathew 2003). Therefore, repeated migraine attacks over the years probably have cumulative adverse consequences on the function of the trigeminovascular pathway to develop central sensitization. The threshold for a central trigeminovascular neuron leads to a state of sensitization depends on the balance between incoming nociceptive signals and their modulation by spinal and supraspinal pathways (Bernstein and Burstein 2012).

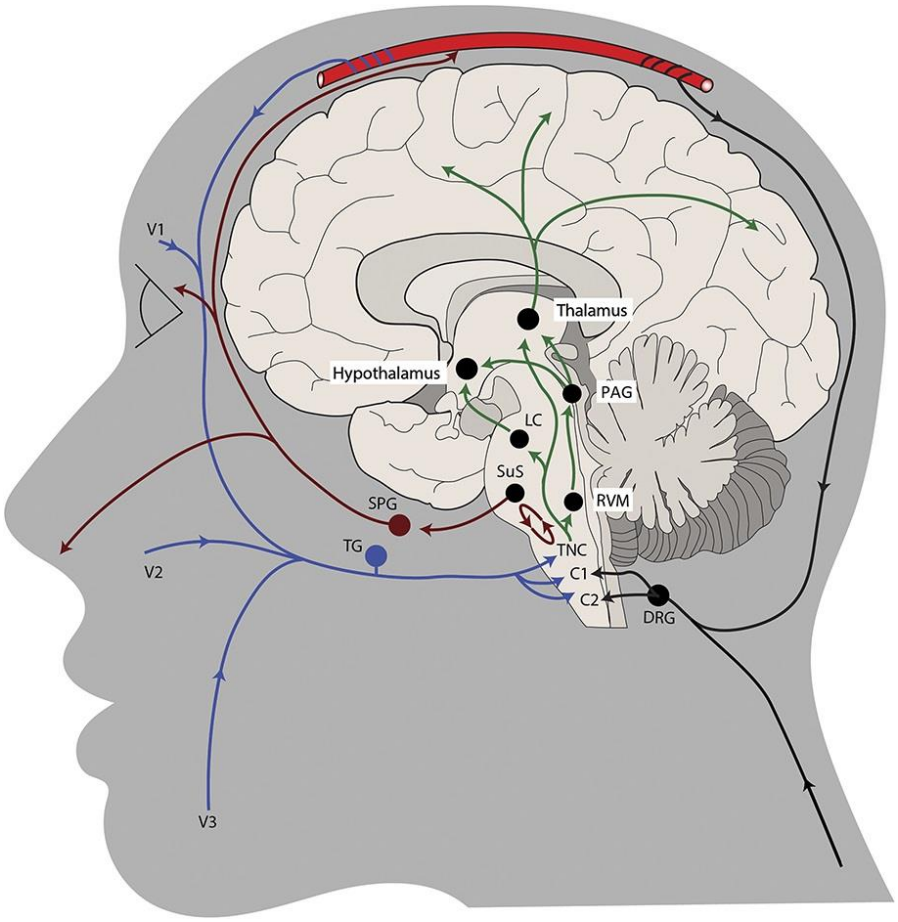

Figure 2. Ascending pathways and main structures in migraine mechanisms. The ascending axonal projections of trigeminovascular TCC neurons consisting of the trigeminal nucleus caudalis (TNC) and the C1 and C2 contributions of the upper spinal dorsal horn convey monosynaptic nociceptive signals to brainstem, hypothalamic and thalamic neurons. Figure taken from (Chan et al. 2019) with kind permission of Wolters Kluwer Health.

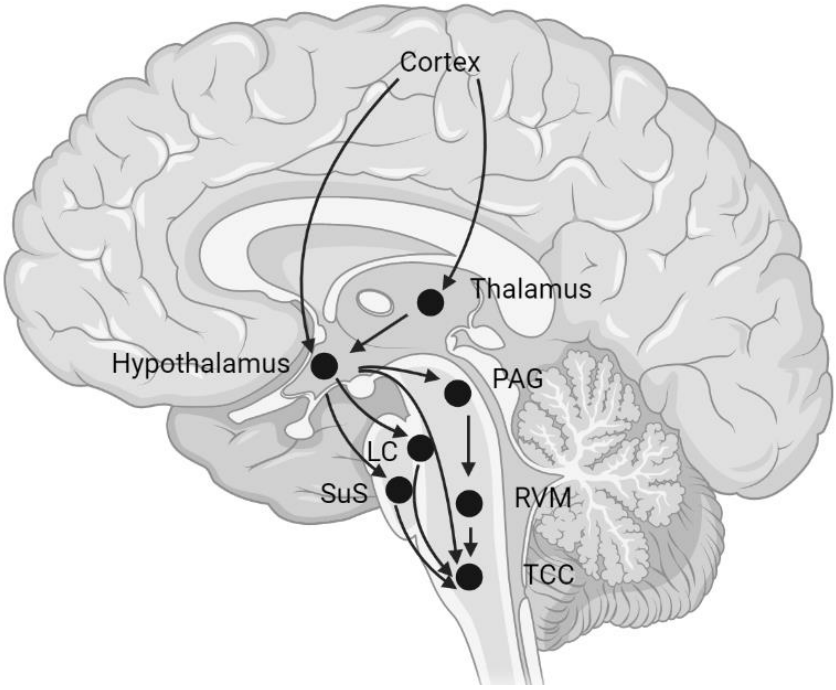

Figure 3. Descending pathways and main structures in migraine mechanisms. TCC: trigeminal cervical complex; LC: locus coeruleus; PAG: periaqueductal gray matter; SuS: superior salivary nucleus; RVM: rostral ventromedial medulla. Figure adapted from (Chan et al. 2019) with kind permission of Wolters Kluwer Health.

1.3 Aura in Migraine

Migraine aura is described as transient neurological symptoms, and refers to a sequence of absolutely reversible visual, sensory or language disturbances, which occur and spread gradually, and either precede or accompany a migraine attack (Charles and Hansen 2015). The International Headache Society (IHS) describes migraine auras as a recurrent attack. The most prevalent aura symptoms are visual disturbances, such as flashes of bright light or wavy, zigzag vision (Viana et al. 2019). Migraine aura usually develop slowly over 5 to 20 minutes, and typically last less than 60 minutes (Viana et al. 2013).

According to the 3rd edition of the International Classification of Headache Disorders (ICHD-3), the diagnosis of migraine without aura need to meet the following criteria (Olesen 2018):

A. At least five attacks fulfilling criteria B–D

B. Headache attacks lasting 4-72 hours (when untreated or unsuccessfully treated)

C. Headache has at least two of the following four characteristics:

1. unilateral location
2. pulsating quality
3. moderate or severe pain intensity
4. aggravation by or causing avoidance of routine physical activity (e.g., walking or climbing stairs)

D. During headache at least one of the following:

1. nausea and/or vomiting
2. photophobia and phonophobia

E. Not better accounted for by another ICHD-3 diagnosis.

The diagnostic criteria for migraine with aura are as follows (Olesen 2018):

A. At least two attacks fulfilling criteria B and C

B. One or more of the following fully reversible aura symptoms:

1. visual
2. sensory
3. speech and/or language
4. motor
5. brainstem
6. retinal

C. At least three of the following six characteristics:

1. at least one aura symptom spreads gradually over 5 minutes
2. two or more aura symptoms occur in succession
3. each individual aura symptom lasts 5–60 minutes
4. at least one aura symptom is unilateral
5. at least one aura symptom is positive
6. the aura is accompanied, or followed within 60 minutes, by headache

D. Not better accounted for by another ICHD-3 diagnosis.

The lifetime prevalence of migraine with aura is around 5% (male/female ratio 1:2). By comparison, migraine without aura, which is approximately 8% (male/female ratio 1:7) (Rasmussen and Olesen 1992). A population-based study showed that the lifetime prevalence of migraine with aura is 3.4% of men as opposed to 7.4% of women (Russell et al. 1995). Similar to the occurrence of migraine attacks, aura afflicts more women than men. Generally, approximately a third of patients with migraine experience aura (Eigenbrodt et al. 2021).

Cortical spreading depression (CSD) is a wave of electrophysiological hyperactivity followed by a wave of inhibition, precisely a slowly propagating wave of near-complete neuronal and glial depolarization across the cortex with a conduction velocity between 2 and 5 mm/min (AAP. 1944; Dodick and Gargus 2008; Harriott et al. 2019; Lauritzen et al. 2011). CSD is now widely thought to contribute to the underlying mechanism of migraine aura (Ayata 2010; Lauritzen 1994), which was described in 1944 by Aristides Leão (AAP. 1944), and also regarded as an intrinsic event possibly causing in migraine headache (Harriott et al. 2019). However, the mechanisms of triggering CSD in the structurally normal, well-nourished cortex of migraineurs remain unknown. An animal study demonstrated that the regional blood flow in the brainstem was transiently increased during spreading depression (Richter et al. 2008). Synaptic drive from subcortical sensory processing structures (brainstem and/or thalamocortical networks) could evoke depolarization of hyperexcitable cortical neurons, which can be sufficient to initiate the regenerative spreading depression process (Vinogradova 2018).

A series of neuroimaging studies support the above opinions. A recent study used combined PET/MR imaging techniques with [^{11}C] PBR28 in migraineurs with visual aura found out increased PET signal in primary visual cortex, visual extrastriate motion-processing areas (second lower tier motion area and middle temporal gyrus), and

Broca's area, which are the areas previously indicated to be involved in CSD generation, as well as in thalamus and primary/secondary somatosensory and insular cortex, which are the areas involved in pain processing (Albrecht et al. 2019). Another neuroimaging study observing changes in the blood oxygenation level dependent (BOLD) signal in patients suffering migraine demonstrated that CSD generates the aura in visual cortex (Hadjikhani et al. 2001).

In addition, brainstem symptoms were included in the definition of migraine with aura in the International Classification of Headache Disorders 3rd edition, which are especially common in familial hemiplegic migraine.

1.4 White Matter Lesion in Migraine

The white matter lesions (WMLs) are clinically mute, mostly progressive microvascular tissue damages (Erdelyi-Botor et al. 2015), which may influence the intrahemispheric size and volume impairment. MRI is a useful diagnostic tool in migraine and shows migraine-related structural brain changes. WMLs are usually discovered on T2-weighted and fluid-attenuated inversion recovery (FLAIR) images. WMLs can negatively affect both physical and cognitive function. Hence, to better carry out management and treatment of migraine, understanding and characterization of the etiology of WMLs in migraineurs are essential (Eikermann-Haerter and Huang 2021).

In general, Migraine patients have two-fold to four-fold increased risk to develop WMLs compared to healthy controls (Eikermann-Haerter and Huang 2021). The reported prevalence of WMLs in migraine varies across studies. Age, presence of aura, resistance to treatment, and disease duration are considered as a risk factor for the development of migraine-related brain WMLs (Negm et al. 2018; Trauninger et al. 2011). A serious of studies showed that 12-46% of migraine patients have these white matter lesions, compared to 2-14% of controls (Bashir et al. 2013; Evans and Olesen

2003; Swartz and Kern 2004; Toghae et al. 2015), and the prevalence of these lesions in migraine with aura is higher than in migraine without aura (Kruit et al. 2010; Tehrani 2018). A community-based cohort follow-up study showed women with migraine had a higher incidence of deep white matter hyperintensities (Palm-Meinders et al. 2012).

WMLs can be found in all four lobes involving the deep white matter, the subcortical, the periventricular, basal ganglia and the callosal commissure locations (Chong et al. 2022; Komaromy et al. 2019). The pathogenesis of WMLs in migraine patients remain not fully understood. Neurogenic inflammation has been reported as a mechanism of migraine headache (Malhotra 2016). During neurogenic inflammation, both of platelet aggregation and endothelial changes occur, which may lead to intravascular thrombosis and microinfarction (Crassard et al. 2001; Tzourio et al. 2001). Migraine patients usually had lower endothelial shear stress which was associated with higher white matter lesion volume (Hoogeveen et al. 2019). Additionally, WMLs are considered to appear because of focal axonal and glial cell injuries (demyelination) possibly resulting from microvascular damage, in connection with decreased intracellular energy metabolism due to impairment of mitochondria (Komáromy et al. 2019). It is plausible that axons passing through these lesions might cause GM changes (Rocca et al. 2006). Besides, WMLs could arise from the cumulative impact of repeated episodes of regional cerebral ischemia during migraine attacks (Kruit et al. 2004). Cerebral blood flow and volume decreased in occipital cortex contralateral to the affected visual hemifield for migraine patients during spontaneous visual auras (Cutrer et al. 1998). In addition, during migraine attacks, repeated or prolonged episodes of oligemia may selectively affect the deep white matter and periventricular white matter, which is supplied by deep penetrating arterioles and potentially vulnerable to prolonged hypoperfusion (Bednarczyk et al. 1998; Gladstone and Dodick 2005). Moreover, based on animal experiments, the matrix metalloproteinases are activated during cortical spreading depression, cleaving intercellular proteins and thereby altering the extracellular matrix, which leads to transient breaches of the blood-brain barrier, and

may result in ischemic brain injury (Gursoy-Ozdemir et al. 2004).

While WMLs can appear frightening in migraine patients, generally they are not associated with neurological disturbance. A previous study indicated that migraine patients with a high deep white matter hyperintensity load at baseline did not experience greater change in cognitive function at the 9-year follow-up than those without a high load at baseline (Palm-Meinders et al. 2012). Previously we have studied the possible influence of WMLs on hemispheric cortical thickness and volume in 161 right-handed female migraineurs, however, no statistically significant correlations were found (Komaromy et al. 2019).

1.5 Episodic versus Chronic Migraine

Migraine can be subtyped as episodic migraine (EM) or chronic migraine (CM) according to the frequency of headache days. EM refers to a diagnosis of migraine with frequency of headache occurring on less than 15 days monthly, and can be further subclassified as low frequency (4–7 days monthly) and high frequency (8–14 days monthly) EM (Dermitzakis, et al. 2022). CM is listed as a complication of EM, and is defined as diagnosis of migraine with more than 15 headache days monthly for more than 3 months, of which at least 8 days per month show the features of migraine headache. CM accounts for 1.3 to 2.4% of general population (Castillo et al. 1999; Scher et al. 1998). A longitudinal study found that each year, around 2.5% of EM suppers develop to CM (Bigal, et al. 2008), while another study demonstrated that 26% of CM cases transformed to other headache conditions over a two-year follow-up period (e.g., episodic tension-type headache, or other episodic headache) (Manack et al. 2011).

1.6 Sex Differences of Neuroimaging in Migraine

In healthy adults, the total brain volume reported to be larger from birth in men than in women by around 11%. Males have higher white/gray matter ratio, intra- versus interhemispheric connectivity, and regional cortical and subcortical volumes. When

structural and lateralization differences are independent of sample size, sex can only explain 1% of total variance (Eliot et al. 2021). Furthermore, there are several, documented structural and functional brain differences between men and women, but it is hard to decide which of them is sex hormone-related (DeCasien et al. 2022).

In terms of migraine, besides the epidemiologic evidence of sex differences, there are also structural and functional sex differences in patients suffering migraine, which were supported by brain MRI studies.

Women reported higher unpleasantness in response to painful stimuli, despite similar pain intensity to men (Bartley and Fillingim 2013). The difference may be associated to the observed sex differences in brain regions involved in emotional processing. In some brain regions, female migraineurs showed greater brain activation in response to pain than male migraineurs, such as the amygdala, parahippocampus, basal ganglia, and posterior cingulate cortex (Maleki and Androulakis 2019). A Harvard study indicated that posterior insula and precuneus cortices were thicker in female migraineurs when compared with male migraineurs or healthy controls of both sexes, furthermore, female migraineurs were more deactivated to response to noxious stimulation in the amygdala and hippocampus, while male migraineurs were more deactivated in the contralateral nucleus accumbens (Maleki et al. 2012). Gonadal hormones and psychological mechanisms may contribute to these sex differences in migraine. Another study showed male migraineurs have smaller parahippocampal grey matter volume compared with female migraineurs (Good et al. 2001), and the potential reasons for the observed changes include differences in the response to migraine attacks, and differential gonadal effects on hippocampal function. A voxel-based morphometry (VBM) meta-analysis indicated that patient sample with higher percentage of female migraineurs was linked to decreased gray matter in the right dorsolateral prefrontal cortex (Dai et al. 2015). According to a pediatric migraine study, compared with male migraineurs and healthy controls, female migraineurs had significantly increased cortical thickness in the right

supplementary motor area (SMA), right precuneus, and left primary somatosensory cortex, as well as had significantly bigger volume in the right caudate, right pallidum and bilateral amygdala. Moreover, female migraineurs have greater resting state functional connectivity (rsFC) of the right precuneus with the left putamen, right caudate, left thalamus, and left amygdala, as well as had greater rsFC of the left amygdala with the bilateral thalamus, right SMA, and bilateral anterior midcingulate cortex (Faria et al. 2015).

1.7 Subcortical Structures Related to Migraine

Neuroimaging studies in migraine have demonstrated that there are structural and functional changes in a variety of cortical areas and subcortical structures which have been considered to be pain-processing regions (Wang et al. 2016). Previously, we investigated cortical thickness and volume of bilateral lobes of migraineurs (Komaromy et al. 2019). In this thesis, we explored volumetric alterations in subcortical structures.

1.7.1 Hippocampus

The hippocampus is a small limbic system structure, which is located in the inner (medial) region of the temporal lobe, and is associated with memory consolidation (in particular long-term memory) and spatial navigation, and is also involved in the stress response (Bruce et al. 2017; Chong et al. 2017a), as well as in pain processing, pain-related attention, and anxiety (Liu et al. 2018). Episodic migraine attacks can be considered as repeated stressor.

The hippocampus largely consists of the dentate gyrus (DG), cornu ammonis (CA) fields and the subiculum. DG is an input region, which receives input from the entorhinal cortex. The CA fields of the hippocampus consist of pyramidal cells and are usually subdivided into four regions (CA1–CA4), and often referred to as the parahippocampal gyrus. The subiculum, the dorsal part of the parahippocampal gyrus, is a major output region of the hippocampal formation (Wible 2013).

Some previous animal and human studies indicate that the hippocampus may play a role in pain processing (Chong et al. 2017a; Ezzati et al. 2014; Mutso et al. 2012). An animal study showed that persistent pain could affect the expression of gene c-Fos in the dorsal and ventral hippocampus: CA1, CA3 and DG (Aloisi et al. 2000). These might result in the long-term volumetric change in the hippocampal substructures. A study by Harvard University reported that structural and functional changes of hippocampus might be the result of repeated stress (Maleki et al. 2013): there were larger hippocampal volume in migraineurs with low frequency (LF) of migraine attacks compared to those with high frequency (HF) of migraine attacks or healthy control groups, and the LF migraineurs had significant volumetric declining trend following the increased number of total attacks. The reason of these results could be interpreted as initial adaptive plasticity of the hippocampus (Glasper et al. 2012). Meanwhile, the same study (Maleki et al. 2013) also found significantly decreased functional connectivity between hippocampus and other brain regions (contralateral supra marginal gyrus, bilateral temporal pole, contralateral fronto-orbital, bilateral nucleus accumbens, bilateral anterior insula, bilateral middle frontal and contralateral paracingulate gyruses) in HF migraine group compared to LF migraine group in response to noxious stimulation.

1.7.2 Thalamus

Thalamus is a large egg-shaped mass of grey matter located in the dorsal part of the diencephalon. It is served as a key nociceptive relay station with nerve fibers not merely projecting out information to the cerebral cortex but also receiving feedback information from these multiple cortical areas, such as the somatosensory, motor, visual, auditory, olfactory and limbic regions (Yen and Lu 2013). It is a central area for the processing and integration of pain stimuli, and it has an important role in allodynia, central sensitization, and photophobia in migraine (Younis et al. 2019). Additionally, thalamo-cortical transmission is constantly modulated by different pathways involved

in emotion, cognition, and autonomic responses (Noseda et al. 2014; Noseda et al. 2011; Puledda et al. 2017).

Several studies have indicated structural and functional thalamic changes in migraineurs. An fMRI study found that patients with episodic migraine without aura had increased functional connectivity between the right thalamus and several contralateral brain regions, including superior parietal lobule, insular cortex, primary motor cortex, supplementary motor area and orbitofrontal cortex, and decreased functional connectivity between the right thalamus and some ipsilateral brain areas, including primary somatosensory cortex and premotor cortex (Amin et al. 2018). A morphological study found that migraine patients had smaller thalamus on both sides compared to healthy controls (Naguib et al. 2021). Furthermore, another study found that chronic migraine patients had larger left thalamus when compared to episodic migraine patients (Chen et al. 2020).

1.7.3 Amygdala

The amygdala is one of the two almond-shaped groups of nuclei located in the medial temporal lobe, and has been considered to play a role in the processing of memory, emotion and decision-making (Amunts et al. 2005; Costafreda et al. 2008). An animal study indicated that cortical spreading depression could influence the activity of amygdala (Dehbandi et al. 2008). In addition, structural changes and resting‐state functional changes were seen in emotional processing regions, including the amygdala (Nyholt et al. 2017). Furthermore, the amygdala has been reported as an important structure of the brain for the emotional-affective dimension of pain and pain modulation (Chen et al. 2017b; Thompson and Neugebauer 2017). Moreover, the lateral and capsular part of amygdala probably play a critical role in migraine because both the mediodorsal thalamus and the trigeminal nucleus caudalis are connected with amygdala (Ashina and Geppetti 2015).

A resting-state fMRI research showed that there was an increased connectivity between the amygdala and some brain structures including the anterior insula, secondary somatosensory cortex and thalamus in migraineurs, when compared with health controls. By contrast, patients with trigeminal neuralgia or carpal tunnel syndrome had no significant difference compared to healthy controls, suggesting a relatively specific dysfunctional neurolimbic pain network in migraine (Hadjikhani et al. 2013). Another resting state fMRI study that investigated 351 salience intra-network connectivities in chronic female migraineurs found that the connectivity between bilateral amygdala were significantly decreased, and the overall salient network circuitry desynchronization was shown to be concentrated on the extended amygdala (Androulakis et al. 2018). In addition, significantly higher blood oxygen level–dependent signal intensities in the amygdala of migraineurs were observed during headache attack (Stankewitz and May 2011).

1.7.4 Hypothalamus

The hypothalamus is a small almond sized brain region that is below the thalamus and behind the optic chiasm. As an important part of the autonomic and endocrine regulation, the hypothalamus has been considered to play a crucial role in migraine attack generation (May and Burstein 2019). Connectivity between the hypothalamus and the trigeminal nociceptive pathway has been verified in both preclinical and clinical studies (Kagan et al. 2013; Schulte and May 2016). A PET study found significant activation in hypothalamus during the migraine headache (Denuelle et al. 2007). A diffusion tensor imaging (DTI) study found that migraine patients without aura between attacks had significantly higher mean diffusivity, axial diffusivity, and radial diffusivity values within the hypothalamus, in comparison to healthy control group (Porcaro et al. 2021). A recent DTI/fMRI study indicated that migraine patients had significantly altered diffusivity metrics mainly within posterior hypothalamus, and higher fractal dimension (FD) values in the salience network (SN). Moreover, a positive correlation of the hypothalamic axial diffusivity with migraine severity and FD of SN was revealed.

Additionally, DTI metrics of bilateral anterior hypothalamus positively correlated with the mean attack duration. Therefore, plastic structural changes in the hypothalamus may related to the multidimensional neurocognitive processing of pain that SN is involved in (Porcaro et al. 2022).

1.7.5 Brainstem

The brainstem contains a network of descending circuitry that can modulate nociceptive processing involving the PAG, RVM and spinal trigeminal nucleus (SpV) (Mills et al. 2021). Descending control includes pathways originating in midbrain and brainstem regions and projecting to the spinal cord, which have been considered as key links in the multiple neuronal networks that interact to engender the pain experience (Bannister and Dickenson 2017).

Dysfunction in brainstem descending modulatory circuits may contribute to the onset of migraine (Bee and Dickenson 2009; Moulton et al. 2008). Weiller and his colleagues (1995) carried out a PET study, which included 9 migraine patients without aura. They found that brainstem exhibited increased cerebral blood flow during spontaneous migraine attacks, and the activation persisted even after an injection of sumatriptan, which induced complete relief from migraine attack. They suggested that the pathogenesis of migraine is associated to an imbalance of regulating antinociception in brainstem nuclei. In a later series of studies, the higher spatial resolution of MRI has been applied in migraine research. Researchers further presumed PAG might play the role of “generator” of migraine attacks based on the results from MRI studies (Chen et al. 2016; Welch et al. 2001). However, some experts have different opinions on this. Schulte and May (2016) described a migraine patient who underwent functional MRI every day for 30 days. Finally, they drew a conclusion that the real driver of attacks might be the functional changes in hypothalamo–brainstem connectivity. Although the role of PAG as migraine generator is still debated, the importance of PAG as a modulator of pain perception has been well documented. PAG is at the center of

powerful descending antinociceptive neuronal network (Smith et al. 1994; Welch et al. 2001), which may impair the descending pain modulation (Chen, et al. 2017c). An animal study concluded that focal brain stimulation in PGA can induce profound analgesia (Reynolds 1969). The result can also be reproduced in the human subjects (Mayer 1984; Young and Brechner 1986).

In migraine patients, several PET studies demonstrated increased activation in dorsal pons and dorsal rostral brainstem during the migraine attack phase (Afridi et al. 2005; Bahra et al. 2001). Marciszewski and colleagues (2018) found lower grey matter volumes in migraineurs in the right spinal trigeminal nucleus caudalis, medullary raphe, dorsal medulla, dorsolateral pons and dorsomedial pons. In addition, increased mean and axial diffusivities occurred in migraineurs in the region of the medullary raphe/spinal trigeminal nucleus, dorsolateral pons and PAG. Moreover, an fMRI study found increased infra-slow oscillatory activity in brainstem and increased connectivity strengths and regional homogeneity in midbrain during the period right before migraine attack. However, these resting oscillatory and connectivity changes did not appear after or between migraine attacks (Meylakh et al. 2018).

1.8 Imaging Study of Migraine

1.8.1 Functional Study

Migraine is a mainly functional neurological disorder. Hence, fMRI is a very useful tool to study the underlying mechanisms of migraine. Currently, two main fMRI sequences have been used to investigate migraine brain function: task-based fMRI, and resting-state fMRI. Both sequences are based on recording the BOLD signals that indirectly reflects the variation in the neural activity through changes in the hemoglobin oxidative condition in the vascular bed encircling the activated neural tissue. fMRI studies consistently show that migraine is associated with atypical brain activation in response to various stimuli and atypical functional connectivity. Different fMRI manifestations

can be seen from the migraineurs in different phases of migraine (Al Harrach et al. 2020; Messina et al. 2022; Schwedt et al. 2015). Some results from previous fMRI studies have been mentioned above, we are not going to make a further explanation here, considering also that our present thesis is mainly focused on structural changes in migraine.

1.8.2 Morphological Study

Previously, brain regions were usually segmented manually, and manual segmentation is also considered as the gold standard for accuracy. However, this method is subjective and extremely time-consuming. A segmentation for the whole brain could take days, and also prone to errors (Perlaki et al. 2017). Therefore, automated segmentation tools will be a good alternative for medium and large-scale studies.

Surface Based Morphometry (SBM) and VBM are the two most widely used methods for estimating brain structures in various disease, mainly by using T1-weighted MRI images. SBM and VBM share some common processes: the preprocessing step, and tissue segmentation, spatial normalization, smoothing of the normalized image, and statistical analysis (Goto et al. 2022; Palaniyappan and Liddle 2012). FreeSurfer and FSL are two most commonly used software for brain segmentation. Considering that FSL don't provide cortical parcellation (we had done a previous study for cortical thickness in migraineurs), and the subcortical segmentation is more extensive in FreeSurfer than in FSL (Quilis-Sancho et al. 2020), so we chose FreeSurfer for our segmentations. Moreover, a previous study indicated the results of hippocampal volume from FreeSurfer were more similar to manual hippocampal segmentation than from FSL-First (Pardoe et al. 2009).

1.9 FreeSurfer

FreeSurfer is an open-source software suite developed by Athinoula A. Martinos Center for Biomedical Imaging, which comprises two main processing streams (Gronenschild

et al. 2012): surface-based stream and volume-based stream. The surface-based stream is developed to derive the white and pial surfaces to calculate the cortical volumes and cortical thickness. The surface-based stream starts with separating the two hemispheres, there are three stages from the FreeSurfer cortical analysis pipeline, which includes skull stripped image, white matter segmentation and surface between white and gray and between gray and pia overlaid on the original volume (Dale et al. 1999; Fischl et al. 1999).

The volume-based stream is developed to assign a neuroanatomical label to each (sub)cortical voxel. The stream utilizes a subject-independent probabilistic atlas that is automatically generated from a training set consisting of multiple hand-labeled atlas images. After we perform a high dimensional nonlinear volumetric alignment of the target image to a common atlas space, the region-of-interest labels are automatically assigned to each voxel through a segmentation that maximizes the probability of the input data according to the prior probabilities from the training set (Srinivasan et al. 2020). The stream mainly consists of following stages: affine transformation to the MNI305 space, initial volumetric labeling, intensity normalisation, high dimensional nonlinear volumetric alignment to the MNI305 atlas and labeling of cortical and subcortical structures (Fischl et al. 2002; Fischl et al. 2004).

In general, FreeSurfer pipeline can be broken down into three stages (-autorecon1, 2 and 3): image preprocessing, automatically segmenting the brain, and parcellating the cortex. Each stages contain different steps. Autorecon Processing Stages (https://surfer.nmr.mgh.harvard.edu/fswiki/recon-all) has been illustrated by Figure 4. The volume of cortical structures is extracted with “aparcstats2table” command, and the volume of subcortical structures is extracted with “asegstats2table” command.

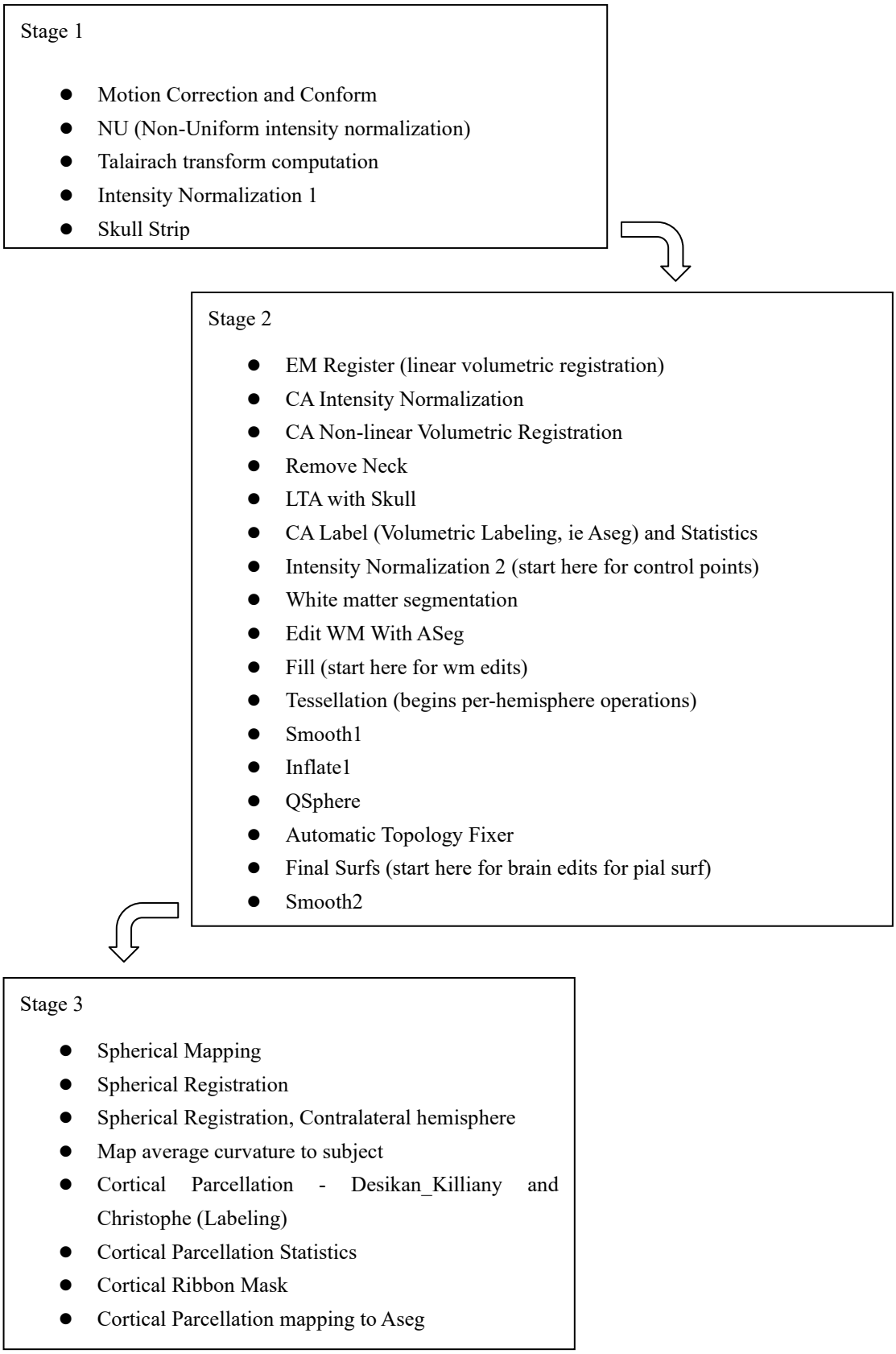

Figure 4. Three stages of FreeSurfer pipeline

After the subject to be processed undergone the general FreeSurfer recon-all pipeline,

additional commands could be run to perform hippocampus and brainstem subfield segmentation via hippocampus subfield module and brainstem subfield module (Iglesias et al. 2015a). Using these modules, the head, body, and tail of left and right hippocampus (Figure 5), as well as medulla, pons, midbrain and superior cerebellar peduncle (SCP) of brainstem, can be defined.

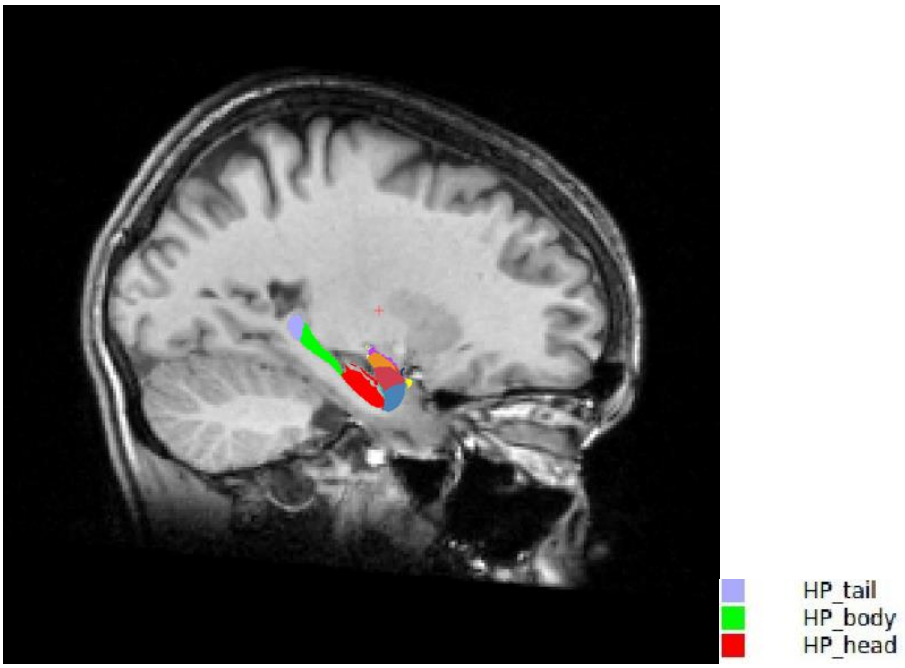

Figure 5. Segmentation of hippocampal subfields and nuclei of the amygdala by FreeSurfer. HP: hippocampal.

2. Research Background and Objectives

Previous studies demonstrated volume changes in subcortical structures in migraine. However, findings are partly inconsistent. Structural changes can be sexually dimorphic in migraine that may have an impact on previous studies examining combined male and female groups. Therefore, only females were included in the present study.

Part I The Volume of the Thalamus and Hippocampus in a Right-Handed Female Episodic Migraine Group

Based on the current literature, some subcortical structures may be functionally impaired in migraine, thereby we hypothesized that WMLs, aura and the migraine characteristics (disease duration and frequency) may affect the volume of thalamus and hippocampus. To test our hypothesis, we measured the volumes of the thalami and hippocampi of migraine patients based on brain MRI and investigated the potential role of WMLs and the migraine characteristics on volumetric changes.

Part II Volumetric Alteration of Brainstem in Female Migraineurs With and Without Aura

It was reported that there are white matter microstructural differences between migraine patients with aura (MwA) and migraine patients without aura (MwoA), highlighting the importance of taking the aura into consideration. Moreover, none of the previous studies investigated the influence of aura on brainstem volume specifically. We hypothesized that the aura may cause brainstem volume changes in migraineurs as an independent risk factor. For that reason, we investigated migraine patients subdivided into two groups (MwA and MwoA), when assessing the effects of migraine on the volumes of the whole brainstem and its subfields.

3. Patients and Methods

3.1 The Volume of the Thalamus and Hippocampus in a Right-Handed Female Episodic Migraine Group

3.1.1 Subjects

A total of 161 females with episodic migraine (mean age 39.3 ± 12.5, range 18-73 years; disease duration 15.6 ± 11.9, range 1-57 years; attack frequency/month 5.6 ± 4.5, range 0.2-20.0; 63 with aura; 52 with lesions; Table 1) were prospectively enrolled to this study. As controls, 40 age-matched healthy female subjects were included (mean age 38.3 ± 10.0, range 19-66 years). Migraineurs had no other types of headaches. None of the included migraine patients' headaches or auras were unilaterally side-locked in nature but showed right hemisphere dominance in 68% (right-sided or bilateral with right>left intensity). MRI was performed during a headache-free period for each patient, the minimum headache-free period was 1 day after the postdrome headache phase. All migraineurs and controls reported right-handedness. All subjects were strictly investigated for medical comorbidities (e.g., past and current medical history, physical examination, routine and autoimmune blood tests, cardiovascular monitoring, BMI, sleep disease, hypertension, gynecological disease, thyroid gland, and cognitive dysfunction) and only those were enrolled in this study who lacked any medical diseases. Brain MRI studies of healthy participants did not show any structural abnormalities.

3.1.2 MRI Acquisition

All subjects were scanned on the same 3T MRI scanner (Magnetom TIM Trio, Siemens AG, Erlangen, Germany) using a 12-channel head matrix coil. Whole-brain T1-weighted three-dimensional axial magnetization-prepared rapid gradient-echo (3D MPRAGE) sequence was acquired using the following parameters:

TR/TI/TE=1900/900/3.4 ms; BW=179 Hz/px; flip angle=9°; FOV=210×240 mm2, matrix size=224×256, slice thickness=0.94 mm, 176 axial slices, 0.94×0.94×0.94 mm3 isotropic voxels.

Beyond the routine T1- and T2-weighted measurements the scanning protocol also included FLAIR imaging (TR/TI/TE=13200/2600/100 ms; BW=401 Hz/px; echo trains=14; FOV=186×220 mm2, matrix size=162×192, slice thickness=1.5 mm, 100 axial slices).

3.1.3 MR Image Analysis

Left and right thalamus segmentation was performed on the high-resolution three-dimensional 3D MPRAGE images using Freesurfer 5.3 image analysis suite (https://surfer.nmr.mgh.harvard.edu/fswiki). The technical details were described previously (Fischl et al. 2002). Quality control was performed throughout the automatic processing stream. When the reconstruction was inaccurate, error correction was performed based on the recommended workflow (http://surfer.nmr.mgh.harvard.edu/fswiki/RecommendedReconstruction). The left and right hippocampus and their subfields (head, body, and tail) were segmented using the development version of FreeSurfer on August 31st, 2017 (https://surfer.nmr.mgh.harvard.edu/fswiki/DownloadAndInstall). This new hippocampal subfield segmentation method is based on a novel statistical atlas built primarily upon ultra-high resolution (~0.1 mm isotropic) ex vivo MRI data. This ex vivo atlas outperforms the in vivo counterpart that was distributed with Freesurfer 5.3 and yields subregion volumes that agree better with those from histological studies. The technical details of this new method were described earlier (Iglesias et al. 2015b).

3.1.4 Analysis of White Matter Hyperintensities

WMLs were considered if visible as hyperintensity on T2-weighted and FLAIR MRI but without low signal intensity lesion on T1-weighted MRI and larger than 3 mm,

appearing in at least two consecutive slices (Figure 6). Supratentorial WMLs were delineated manually on the FLAIR images using 3D Slicer software (http://www.slicer.org, Version 4.6.2, Figure 7). Total and lobar hemispheric WML numbers/volumes were calculated for all patients with white matter lesions (L+). The borders of lobes were defined as previously described (Komaromy et al. 2019).

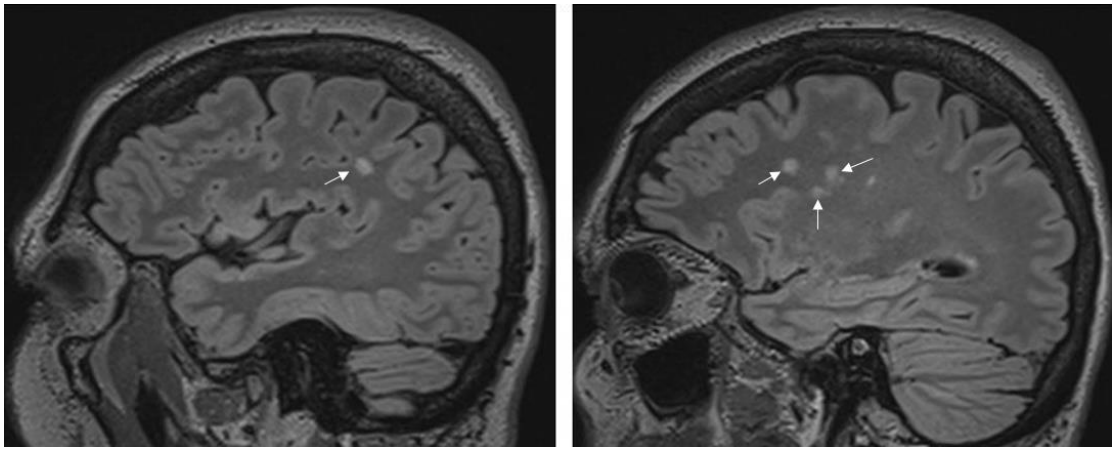

Figure 6. Migraine patient with intrahemispheric white matter lesions. The sagittal fluid-attenuated inversion recovery (FLAIR) images belong to one patient and show high signal intensities in the white matter. The arrows show the lesions.

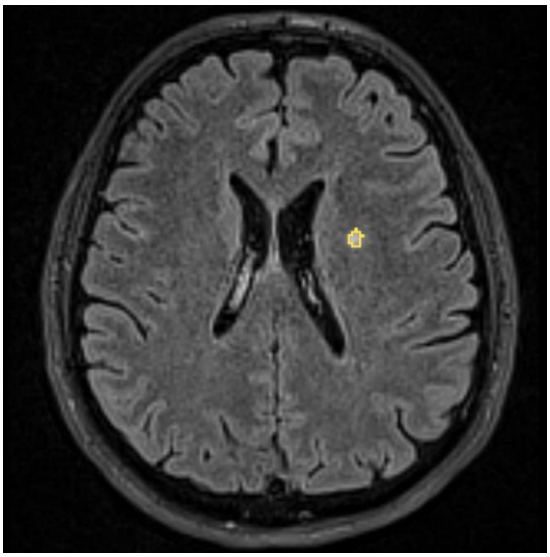

Figure 7. Delineation of a white matter lesion in a female migraineur. An example of an axial FLAIR image used to assess white matter lesion is shown in the background. Yellow indicates the outline of a manually delineated white matter lesion.

3.1.5 Statistical Analysis

Statistical analyses were performed using SPSS 20.0 software (IBM Corp., Armonk, NY, USA).

Differences in age between the whole migraine group and healthy controls were assessed by the Mann–Whitney U-test. Age differences among migraine subgroups (L+ and L-: patients without white matter lesions) and the healthy control group were assessed by the Kruskal–Wallis test followed by the Mann–Whitney U-test. The age difference between migraine patients without aura (A-) and with aura (A+) was assessed using Mann–Whitney U-test.

Wilcoxon signed-rank test was used to identify whether there were statistically significant differences between the total volume of the left and right hippocampus, as well as between left and right thalamus, separately in the healthy control group and the migraine group. Volume differences between the healthy control group and the migraine group in left and right hippocampus, as well as thalamus, were assessed by analysis of covariance (ANCOVA) using age and total intracranial volume (ICV) as covariates.

Differences in continuous migraine-related variables (i.e., disease duration, migraine attack frequency) between migraine subgroups (L+ and L-) were compared using the Mann–Whitney U-test, while differences in the rate of aura (A+ or A-) between the same subgroups were assessed using the Fisher's exact test. Within the migraine group with lesions (L+), Wilcoxon signed-rank test was used to test for differences between left- and right hemispheres in total and lobar hemispheric WML numbers/volumes. These differences were not tested for the temporal and occipital lobes, because lesions were rare in these lobes with a median lesion number of 0.

Differences in the thalamus, hippocampal, and hippocampal subfield volumes among the three groups (L+, L-, control) were assessed using ANCOVA with age and ICV as

covariates. In the case of significance, the least significant difference (LSD) post hoc analyses were conducted for pairwise comparison of the three groups.

Within the whole migraine group, the same volumes were compared between patients with aura (A+) and without aura (A-) using ANCOVA with age and ICV as covariates. The possible effects of other migraine characteristics (i.e., disease duration, migraine attack frequency) on the examined volumes were tested using stepwise multiple linear regression analyses. In these models age, ICV, lesion, and aura group variables were also included as possible predictors.

Within the migraine group with lesions (L+), we assessed the potential relationships between left and right hemispheric brain structure volumes and the total and lobar lesion numbers/volumes in the corresponding hemisphere. Since the lesion number/volume data were not normally distributed (right-skewed with several extreme values), in order to perform powerful statistical analyses, the effects of lesion numbers/volumes were assessed by creating binarized subgroup variables based on the median split of them: e.g., the subgroup with a low number/volume (below or equal to the median) vs. subgroup with a high number/volume of left hemispheric frontal lobe lesions. The effects of these subgroup variables on the examined volumes were tested using stepwise multiple linear regression analyses including all these subgroup variables, age, and ICV as possible predictors. Since lesions were rare in the temporal and occipital lobes with a median lesion number of 0, the effects of lesion number/volume in these lobes were not assessed. Results were considered significant at $p \leqslant 0.05$.

3.2 Volumetric Alteration of Brainstem in Female Migraineurs With and Without Aura

3.2.1 Subjects

A total of 161 female patients fulfilling the IHS classification criteria (Bes et al. 2013)

for migraine were initially screened from the Outpatient Headache Clinic of the Department of Neurology, Medical School, University of Pécs, Hungary. All migraineurs included in this study had recurrent headaches, and none of them were on chronic prophylactic therapy before receiving brain MRI scan. To exclude possible disturbing effects of macroscopic white matter lesions on morphometric brain changes, fifty-two patients with visible WMLs were excluded. In addition, 19 further migraine patients were excluded because of inadequate quality of MR images (i.e., low signal intensity of the inferior part of image due to poor slab profile of slice selective excitation). Eventually, 90 migraine patients were investigated in this study.

The demographic and clinical data of migraineurs were the following: mean age 37.4 ± 11.6, range 18–73 years; disease duration 14.0 ± 11.0, range 1–43 years; attack frequency/month 5.6 ± 4.4, range 0.5–17.0; 61 migraineurs without aura, 29 migraineurs with aura. Migraineurs had no other types of headaches. All migraine patients' headache or aura were not unilaterally side-locked. An MRI was performed in a headache-free period for each patient. All migraineurs were right-handed based on self-report.

As for the healthy controls, 40 age-matched healthy female subjects were initially enrolled, but only 32 of them (mean age 38.0 ± 10.5, range 19–66 years) were finally included because of the inadequate image quality (i.e., low signal intensity of the inferior part of the image). Similar to migraine patients, all healthy controls were right-handed. All control subjects were free of headache, and their brain MRI studies did not show any structural abnormalities. Migraineurs and controls were free of any medical comorbidities.

3.2.2 MRI Acquisition

All subjects were scanned on the same 3T MRI scanner (Magnetom TIM Trio, Siemens AG, Erlangen, Germany) using a 12-channel head matrix coil. Beyond the routine T1-

weighted, T2-weighted and FLAIR imaging, whole-brain T1-weighted 3D MPRAGE sequence was also acquired using the following parameters: TR/TI/TE=1900/900/3.4 ms; BW=179 Hz/px; flip angle=9°; FOV=210×240 mm2, matrix size=224×256, slice thickness=0.94 mm, 176 axial slices.

3.2.3 MR Image Analysis

Brainstem segmentation was performed on the 3D MPRAGE images using the development version of FreeSurfer image analysis suite released on August 31st, 2017 (https://surfer.nmr.mgh.harvard.edu/fswiki/DownloadAndInstall). The technical details were described previously (Fischl et al. 2002). Quality control was performed throughout the automatic processing stream. When the reconstruction was inaccurate, error correction was performed based on the recommended workflow (http://surfer.nmr.mgh.harvard.edu/fswiki/RecommendedReconstruction). Freesurfer is based on an algorithm performing automated segmentation and labels the brainstem into its four main subfields (medulla, pons, midbrain and SCP: superior cerebellar peduncle). Given that SCP is not strictly part of the brainstem, we did not investigate it in this study.

3.2.4 Statistical Analysis

Statistical analyses were performed using SPSS 26.0 software (IBM Corp., Armonk, NY, USA). Differences in age between the whole migraine group and healthy controls were assessed by the Mann–Whitney U-test. Age differences among migraine subgroups (MwA and MwoA) and the healthy control group were assessed by Kruskal–Wallis test followed by pair-wise Mann–Whitney U-test. Differences of continuous migraine related variables (i.e., disease duration and migraine attack frequency) between migraine subgroups (MwA and MwoA) were compared by Mann–Whitney U-test.

Differences in brainstem and brainstem subfield volumes between whole migraine

patients and healthy controls, as well as among our three groups (MwA, MwoA and healthy control), were assessed by using ANCOVA with age and total ICV as covariates. In case of statistical significance, LSD post-hoc analyses were conducted for pairwise comparison of the three groups. Considering that MwoA group was significantly older than MwA group, a closely age-matched subset of 24 MwoA and 24 MwA was established (±2 years difference for each pair of patients), and also analyzed by ANCOVA.

The possible effects of other migraine characteristics (i.e., disease duration, migraine attack frequency, aura, age and ICV) on the examined volumes were tested by stepwise multiple linear regression analyses. To control Type I error and achieve great statistical power, the Bonferroni correction was applied for omnibus tests with the number of different brainstem subfields (n=3). Given that the familywise Type I error probability for LSD procedure remains in the nominal alpha level when comparing three groups (Seaman et al. 1991), it was not necessary for correcting in our post hoc tests (Goeman and Solari 2022). Results were considered significant when p-values were less than 0.05.

4. Results

4.1 The Volume of the Thalamus and Hippocampus in a Right-Handed Female Episodic Migraine Group

There was no significant difference in age between the whole migraine and the control groups (p=0.736). The Kruskal–Wallis test revealed significant age differences among migraine subgroups (L+ and L-) and controls (p=0.001). Post hoc testing indicated that the L+ subgroup was significantly older than the other two groups (Table 1). There was no significant difference in age between patients without aura (A-) and with aura (A+) (p=0.816, 2-sided exact p-value).

Table 1. Demographic and clinical data of migraine patients and healthy controls

	Lesion+ (n=52)	Lesion- (n=109)	Controls (n=40)	Differences (p-value) L+ vs. L-	L+ vs. C	L- vs. C
Age (years)	44.6 ± 13.1 (20-72)	36.7 ± 11.4 (18-73)	38.3 ± 10.0 (19-66)	<0.001[a]	0.018[a]	0.422[a]
Disease duration (years)	19.8 ± 12.9 (1-57)	13.7 ± 10.9 (1-43)	-	0.003[a]	-	-
Migraine attack frequency/month	5.4 ± 4.2 (0.2-17.0)	5.7 ± 4.6 (0.5-20.0)	-	0.807[a]	-	-
Patients with aura	31 (59.6 %)	32 (29.4 %)	-	<0.001[b]	-	-

Lesion+: Migraineurs with lesions; Lesion-: Migraineurs without lesions; Values are given as mean ± standard deviation (minimum-maximum).

[a]Mann–Whitney U-test, (2-sided exact p-value)

[b]Fisher's exact test, (2-sided exact p-value)

The Wilcoxon signed-rank test showed that the left hippocampus had a smaller total volume when compared with the right hippocampus in both the healthy control group (3353±252 mm3 vs. 3517±289 mm3; Z=-4.968, $p < 0.001$) and the migraine group

(3379±293 mm3 vs. 3494±317 mm3; Z=-8.653, p < 0.001). On the other hand, the left thalamus had a bigger volume when compared with the right thalamus in both the healthy control group (7708±747 mm3 vs. 6984±562 mm3; Z=-5.275, p < 0.001) and the migraine group (7675±817 mm3 vs. 6951±689 mm3; Z=-10.835, p < 0.001). There were no significant differences between healthy control group and the whole migraine group in the total volume of the left hippocampus (p=0.703), right hippocampus (p=0.436; one outlier patient was excluded based on standardized residuals>3), left thalamus (p=0.801, one outlier patient was excluded based on standardized residuals<−3), and right thalamus (p=0.726).

Disease duration and the rate of aura were significantly higher in L+ patients, while migraine attack frequency was not significantly different between L+ and L- subgroups (Table 1). Results for the number and volumes of supratentorial white matter lesions of L+ patients in different hemispheric lobes and in the total left and right hemispheres are presented in Table 2. Wilcoxon signed-rank test indicated that there were no differences between the left and right hemispheres in total (p=0.341), frontal (p=0.084) and parietal (p=0.264) WML numbers, and no differences were found for the total (p=0.448) and frontal (p=0.226) lesion volumes. However, lesion volume was bigger in the left- compared to the right-hemispheric parietal lobe (Z=-2.097; p=0.036).

Table 2. Number and volumes of supratentorial white matter lesions in the Lesion+ migraine subgroup

Location	Left hemisphere		Right hemisphere	
	Lesion number	Lesion volume (mm^3)	Lesion number	Lesion volume (mm^3)
Total	5 (0-41)	171 (0-4343)	5 (0-39)	153 (0-4632)
	8	355	8.75	354
Frontal lobe	3 (0-24)	68 (0-1542)	3 (0-26)	92 (0-3283)
	5	231	6	180
Parietal lobe	1 (0-11)	31 (0-2558)	0.5 (0-11)	6 (0-1749)
	4	167	3	115
Temporal lobe	0 (0-4)	0 (0-274)	0 (0-6)	0 (0-658)
	1	27	1	21
Occipital lobe	0 (0-2)	0 (0-183)	0 (0-2)	0 (0-374)
	0	0	0	0

Lesion+: Migraine patients with lesions; Values are given as median (minimum-maximum) and interquartile range, separately for each hemispheric lobe and for the total left and right hemispheres.

After correcting for the confounding effects of age and ICV, the patients with lesions (L+) showed significantly smaller right thalamus and right hippocampal tail volumes than patients without lesions (L-) and controls (Table 3). For the right hippocampal body, the ANCOVA showed a strong trend ($p=0.051$), therefore, we performed the post hoc tests, which indicated significantly smaller volume in L+ patients when compared to L- patients and a trend when compared to the control group (Table 3).

Focusing on the migraine group, patients without aura (A-) showed larger right hippocampus, right hippocampal body, and tail than patients with aura (A+) (Table 4).

Table 3. Group differences in the volumes of examined structures among lesion-related migraine subgroups and controls

	Groups			Statistics				
				ANCOVA test		LSD post-hoc test		
	L+	L-	Control	F (df1,df2)	P	L+ vs. L-	L+ vs. C	L- vs. C
L thalamus	7548 (914)	7758 (727)	7708 (747)	1.639 (2,194)	0.197	-	-	-
R thalamus	6698 (689)	7072 (659)	6984 (562)	5.092 (2,195)	0.007	0.002	0.039	0.560
L hippocampus	3340 (345)	3397 (265)	3353 (252)	0.735 (2,195)	0.481	-	-	-
R hippocampus	3434 (364)	3522 (289)	3517 (289)	1.722 (2,195)	0.181	-	-	-
L hippocampal head	1689 (194)	1709 (157)	1685 (124)	0.311 (2,195)	0.733	-	-	-
R hippocampal head	1742 (215)	1756 (156)	1762 (160)	0.288 (2,194) 0.438[a] (2,192)	0.750 0.646[a]	-	-	-
L hippocampal body	1150 (102)	1158 (84)	1140 (80)	0.903 (2,189)	0.407	-	-	-
R hippocampal body	1153 (115)	1191 (94)	1185 (103)	3.022 (2,194)	0.051	0.018	0.064	0.928
L hippocampal tail	504 (70)	524 (62)	518 (67)	1.672 (2,194) 1.776[b] (2,193)	0.191 0.172[b]	-	-	-
R hippocampal tail	539 (71)	567 (67)	570 (56)	3.589 (2,195)	0.029	0.015	0.025	0.766

L+: Migraine patients with lesions; L-: Migraine patients without lesions; ICV: total intracranial volume; L: left; R: right; LSD: least significant difference

Subjects were excluded from the analysis if the corresponding standardized residuals from the ANCOVA model were below −3 or above 3. A maximum of three subjects had to be excluded from either group. Volumes are presented as uncorrected mean (standard deviation) in mm3 for subjects included in the ANCOVA statistics. For post hoc tests P-values are given.

[a]Statistical values are also presented for the group effect at mean ICV when including the significant Group*ICV interaction term in the model.

[b]Statistical values are also presented for the group effect when including the significant Age*ICV interaction term in the model.

Table 4. Group differences in the volumes of examined structures between aura-related migraine subgroups

	Groups		ANCOVA test	
	Aura+	Aura-	F (df1, df2)	P
L thalamus	7655 (942)	7688 (729)	0.295 (1,157)	0.588
R thalamus	6966 (765)	6941 (640)	0.003 (1,157)	0.955
L hippocampus	3339 (328)	3404 (267)	3.004 (1,157)	0.085
R hippocampus	3439 (346)	3529 (293)	4.849 (1,157)	0.029
L hippocampal head	1685 (192)	1714 (153)	1.947 (1,157)	0.165
R hippocampal head	1739 (208)	1759 (154)	1.115 (1,156)	0.293
			1.141[a] (1,155)	0.287[a]
L hippocampal body	1143 (106)	1164 (96)	2.615 (1,156)	0.108
R hippocampal body	1158 (107)	1192 (98)	6.504 (1,156)	0.012
L hippocampal tail	512 (67)	524 (66)	1.654 (1,157)	0.200
R hippocampal tail	542 (63)	569 (71)	6.669 (1,157)	0.011
			6.998[b] (1,156)	0.009[b]

Aura+: Migraine patients with aura; Aura-: Migraine patients without aura; ICV: total intracranial volume; L: left; R: right

Subjects were excluded from the analysis if the corresponding standardized residuals from the ANCOVA model were below −3 or above 3.

Maximum one subject had to be excluded from the Aura- group. Volumes are presented as uncorrected mean (standard deviation) in mm3 for subjects included in the ANCOVA statistics.

[a.]Statistical values are also presented for the group effect when including the significant Age*ICV interaction term in the model.

[b.]Statistical values are also presented for the group effect at mean ICV when including the significant Group*ICV interaction term in the mode.

Within the whole migraine group, neither the disease duration nor migraine attack frequency was selected by stepwise linear regression as significant predictors of the examined structural volumes, except for the volumes of hippocampal tails. Using multiple linear regression including the volume of the left hippocampal tail as the dependent variable and migraine attack frequency, age, and ICV as independent variables, we found a significant negative correlation between attack frequency and the volume of the left hippocampal tail (r=-0.187, p=0.018; Figure 8A). Based on our

results reported in Table 3 and Table 4, for the right hippocampal tail, we also included the lesion (L- vs. L+) and aura (A- vs. A+) in addition to migraine attack frequency, age, and ICV as independent variables in the multiple linear regression model. We found a negative correlation of attack frequency with the right hippocampal tail volume (r=-0.212, p=0.008; Figure 8B).

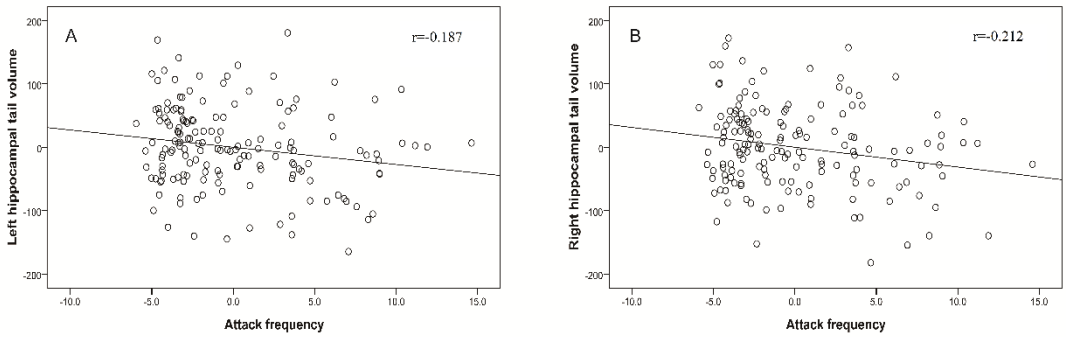

Figure 8. Partial regression plots demonstrating that more frequent migraine attacks were associated with decreased volumes of the left hippocampal tail (after controlling for the effects of age and ICV, Fig. 8A) and the right hippocampal tail (after controlling for the effects of age, ICV, lesion, and aura, Fig. 8B).

In the L+ migraine group, none of the binarized total or lobar hemispheric lesion number/volume variables were selected by stepwise linear regression as significant predictors of the examined structural volumes.

4.2 Volumetric Alteration of Brainstem in Female Migraineurs With and Without Aura

There was no significant difference in age between the whole migraine group and healthy controls (2-sided exact p-value=0.810). The Kruskal–Wallis test revealed age differences among the two migraine subgroups (MwA and MwoA) and controls (asymptotic p-value=0.022). Post hoc testing indicated that MwoA group was significantly older than MwA group (p=0.006), but the migraine subgroups were not significantly different in age compared to the control group (Table 5). There was no significant difference in disease duration (2-sided exact p-value=0.113) or migraine attack frequency/month (2-sided exact p-value p=0.761) between migraine subgroups.

Migraineurs as a whole had greater medulla volume (Figure 9B) compared to healthy controls (p=0.002). The volumes were not different between migraineurs and healthy controls in other structures (p=0.761, 0.826, and 0.599 for the pons, midbrain and whole brainstem, respectively). There were no significant age*group, age*ICV or group*ICV interactions in the performed analyses.

When comparing our three groups (MwA, MwoA and HC: healthy controls), only the volume of medulla was found to be significantly different among the groups (p=0.009, Table 6). Post-hoc testing indicated that both MwA and MwoA groups had bigger medulla (Figure 9C) compared to the HC group (p=0.040 and p=0.002, respectively), while the two migraine subgroups were not different in medulla volume (p=0.555). Closely age-matched MwoA and MwA showed no significant difference either (p=0.943, 0.251, 0.971 and 0.836 for whole brainstem, medulla, pons and midbrain volumes, respectively). Focusing on the whole migraine group, the stepwise linear regression showed a significant positive correlation (Figure 10) between disease duration and the medulla volume (r=0.334, p=0.001; no other potential independent variables were included in the model by the stepwise method). For other brainstem structures, no migraine characteristics were chosen by the stepwise method as significant predictors of the volume.

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Table 5. Demographic and clinical data of migraine patients and healthy controls

	Aura+ (*n*=29)	Aura- (*n*=61)	Controls (*n*=32)	Differences (P value)		
				A+ vs. A-	A+ vs. C	A - vs. C
Age (years)	32.4 ± 10.8 (18-50)	39.8 ± 11.3 (18-73)	38.0 ± 10.5 (19-66)	0.006[a]	0.066[a]	0.493[a]
Disease duration (years)	11.4 ± 10.1 (1-33)	15.3 ± 11.3 (1-43)	-	0.113[b]	-	-
Migraine attack frequency/month	5.7 ± 4.6 (5-15)	5.5 ± 4.4 (1-17)	-	0.761[b]	-	-

Aura+/A+: Migraine patients with aura; Aura-/A-: Migraine patients without aura; Controls/C: Healthy controls.

Values are given as mean ± standard deviation (minimum-maximum).

[a.] The statistical P values were based on post-hoc Dunn's test.

[b.] The statistical P values were based on Mann–Whitney U-test (2-sided exact p-value).

Table 6. Group differences in brainstem and subfield volume

	Groups			ANCOVA test	
	Aura+	Aura-	Controls	F(df1,df2)	P (uncorrected)
Brainstem (mm^3)	22276 ± 2008	22407 ± 1793	22119 ± 2180	0.376 (2,117)	0.688
Medulla (mm^3)	3424 ± 448	3552 ± 459	3236 ± 411	4.926 (2,117)	0.009[b]
Pons (mm^3)	13146 ± 1365	13216 ± 1325	13234 ± 1591	0.383 (2,117)	0.683
Midbrain (mm^3)	5455 ± 399[a]	5424 ± 382	5424 ± 509	0.135 (2,116)[a]	0.874[a]

Aura+: Migraine patients with aura; Aura-: Migraine patients without aura; Controls: Healthy controls.

Volumes are presented as uncorrected mean ± standard deviation.

[a] One subject was excluded due to the corresponding standardized residuals from the ANCOVA model were below −3 or above 3.

[b] The uncorrected P-value survive Bonferroni correction.

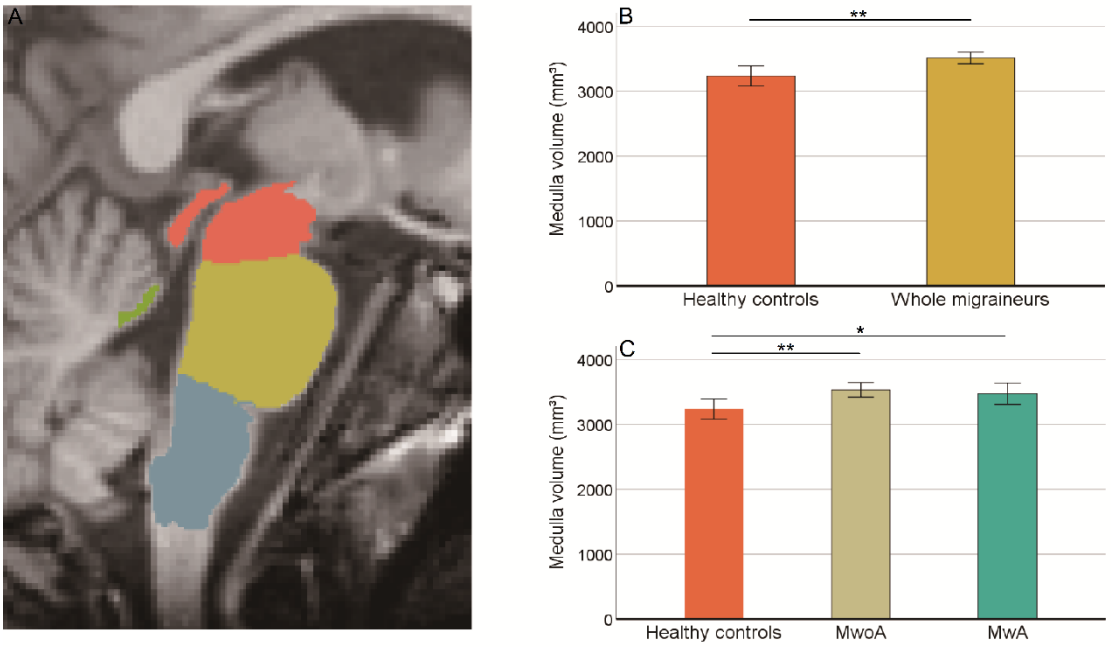

Figure 9. Segmented brainstem and group differences in medulla volume. MwA: Migraine patients with aura; MwoA: Migraine patients without aura. (A) Subfields of brainstem segmented by FreeSurfer. (B) Medulla volume comparison between healthy controls and all migraineurs. (C) Volume comparisons among migraine subgroups and healthy control group. Models adjusted for age and total intracranial volume. The error bars indicate standard error. Significant differences between groups are indicated by asterisk, *: $P < 0.05$; **: $P < 0.01$.

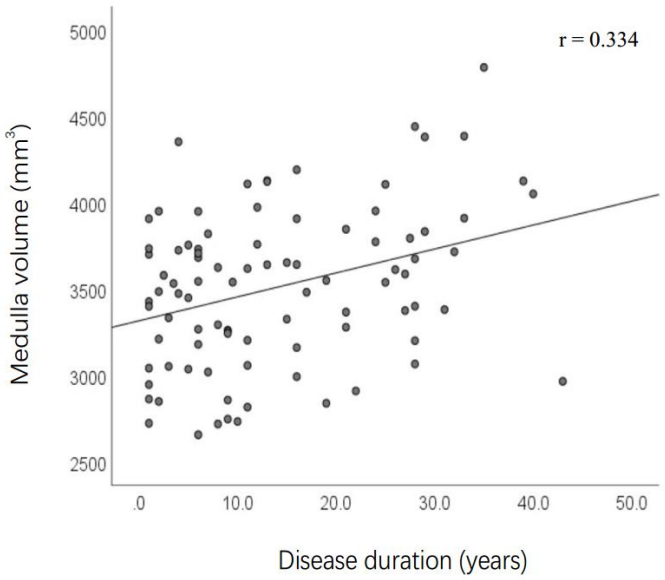

Figure 10. Scatter plot showing that disease duration and medulla volume have a significant positive correlation.

5. Discussion

5.1 The Volume of the Thalamus and Hippocampus in a Right-Handed Female Episodic Migraine Group

In this rather homogenous patient population, we investigated 161 right-handed episodic migraineurs. To eliminate the confounding effect of sex-related differences in the headache characteristics and brain size, only women were enrolled in the study. The total volumes of the left or right thalami and hippocampi were not different between migraineurs and controls. The volume of the right thalamus was smaller than the left one within the migraine and control groups. Patients with WMLs showed a smaller right thalamus than in lesion-negative migraineurs. The hippocampi were smaller on the left side in both groups. The hippocampus subgroup analysis of migraine patients and the subfield volume values of all three groups showed statistically significant differences. The white matter lesions, the presence of aura, and the attack frequency appeared to be risk factors for smaller volumes. It is also possible that the dominant unilateral headache side with more attacks on the right side has a role in the volume differences of the thalami. Since the patients' memory was only reliable for the last month, we didn't use these data for statistical analysis.

5.1.1 Thalamic and Hippocampal Volume Asymmetries in Healthy Subjects

In our right-handed healthy female control group significant volume differences were detected between the thalami (R<L) and hippocampi (R>L) of the two hemispheric sides. Since in the cited morphometric studies (Keller et al. 2012; Szabo et al. 2001; Jang et al. 2017), the participants not separated by sex, and handedness was not documented in one study, these findings are just partly comparable with our data. However, handedness may influence the hippocampus volume on the same side.

5.1.2 Manual Dominance in Migraine

In a large retrospective study, the authors collected 546 patients with migraine aged between 16 and 65 years, reporting the manual dominance according to the Edinburgh

test. They included 466 right-handed and 80 left-handed subjects with migraine and registered 4215 unilateral painful attacks. It was concluded that manual dominance influences the side of pain lateralization in migraine (La Pegna et al. 2021). Considering our one-month long database, the right-handed data are congruent.

5.1.3 The Volume of the Thalamus in Migraine

In a study of 35 patients with migraine without aura and 40 healthy controls, right and left thalamic volumes were not different between the patients and controls. The authors found that right anteroventral and right and left medial geniculate nuclei volumes were significantly increased, but the right and left parafascicular nuclei volumes were decreased in the patients with migraine. The authors concluded that the result might contribute to the underlying pathogenesis of migraine (Shin et al. 2019).

In measurements of thalamic subregions in 77 migraineurs with episodic migraine and 30 controls, selective functional hypoconnectivity in the thalamic subregions was detected (Magon et al. 2015). The data provided neuroimaging evidence of thalamocortical pathway dysfunction in episodic migraine including emotion and personality traits in migraineurs.

In another study, significant volume reductions of three thalamic nuclei were observed in migraineurs: central nuclear complex, anterior nucleus, and lateral dorsal nucleus (Yang et al. 2022). This study indicated structural thalamic abnormalities in patients with migraine. The thalamic nuclei with reduced volumes were connected to the limbic system.

In a recent thalamic study, the volume was investigated by analyzing MRI data obtained from a large study, which specifically included women migraineurs with aura, unrelated migraine-free matched controls, and migraine aura-free co-twins. None of the analyses showed any between-group differences in the volume of the thalamus or of individual thalamic nuclei. These results indicated that the pathophysiology of migraine with aura did not involve alteration of thalamic volumes (Hougaard et al. 2020). According to our

findings, thalamic volume abnormalities were not detected in migraine aura patients in the latter publication, but we found WML associated smaller volume.

5.1.4 The Volume of the Hippocampus in Migraine

Hippocampus and amygdala volumes were measured by 3-T brain MRI in 31 controls and 122 migraine patients who were categorized into eight groups by headache frequency (Liu et al. 2017). Hippocampus volumes fluctuated in patient groups but did not differ from the controls. In migraine patients, after a 2–year long follow-up, the right hippocampus volume was positively associated with a good migraine outcome after adjustment of headache frequency (OR 4.72, $p = 0.024$).

In a review of hippocampal volume, a longitudinal study discovered decreased volume in newly diagnosed migraine patients after 1 year (Liu et al. 2013). A cross-sectional study also suggested an adaptive increase of volume at low headache frequency and a maladaptive decrease of volume at higher headache frequency (Maleki et al. 2013). These results were interpreted as either initial adaptive plasticity of the hippocampus or a larger hippocampus (i.e., a pre-existing condition) in migraineurs that then decreases with increased attack frequency (Glasper et al. 2012). Evidence for the first interpretation has come from animal models on neurogenesis in the structure that is known to persist into adulthood. Stress (e.g., migraine) may mediate adaptive structural plasticity through the remodeling of dendrites and synapses (McEwen and Kalia 2010). With repeated stress involved in migraine attacks including pain, glucocorticoids, cortical spreading depression, and gonadal hormones, elevated and prolonged levels of excitatory amino acids are likely released (Craft et al. 2004; Milde-Busch et al. 2011). Moreover, the aging rat hippocampus displays elevated and prolonged levels of excitatory amino acids released during acute stress. It is probable that structural plasticity in response to repeated stress starts out as an adaptive and protective response but ends up as damage if the imbalance in the regulation of the key mediators is not resolved. It is likely that morphological rearrangements in the hippocampus impair memory functions, and it is conceivable that these may also have a role in chronic pain perception (McEwen 2001). In another study, an investigation of 61 migraineurs and

57 healthy subjects, the authors concluded that migraineurs had smaller hippocampal volume and stronger hippocampal-cortico-limbic connectivity compared to healthy subjects. Hippocampal volumes and measures of hippocampal volume connectivity with other cortico-limbic network regions associated with symptoms of allodynia (Chong et al. 2017a). These studies show hippocampal volume changes and support the role of migraine frequency in the volume abnormalities. Since we also found hippocampal tail volume association with migraine frequency, these findings are consistent with our results.

5.1.5 WMLs and Migraine Characteristics

The thalamic and hippocampal volume changes are likely the consequence of the recurrent migraine headaches and accumulate with migraine years. The WMLs are worsening with longer migraine disease duration, but they can be found at any age of migraineurs. A previous MRI study of supratentorial white matter hyperintensities in migraine patients demonstrated tissue damage with axonal loss, decreased glial cell density with impaired energy metabolism, enlarged extracellular space with an increased extracellular water fraction and decreased blood flow and volume (Aradi et al. 2013). In addition, the reactive oxygen species (ROS) are the contributors of oxidative stress which can cause vascular endothelial dysfunction in migraine and that may lead to tissue damage in WMLs (Erdelyi-Botor et al. 2017).

Both the thalamus and hippocampus are involved in migraine bout-related pain processing which thereby causes stress. These include the headache intensity, frequency, and the attack dominant hemisphere. The pain may have a role in the volume changes, especially in the hippocampi.

Migraine aura is usually unilateral and visual, but it also can be sensory, motor, brainstem and retinal or cause speech/language disturbance. The CSD is a slowly propagated wave of depolarization of neurons and glial cells, followed by a subsequent sustained suppression of spontaneous neuronal activity, accompanied by complex and variable changes in vascular caliber, blood flow, and energy metabolism. Although the

spreading depression (SD) has been most extensively studied in the cortex, the phenomenon may occur in all neural tissues, including hippocampus, cerebellum, and retina, among other regions (Charles and Brennan 2009; Lauritzen 2001). Growing evidence suggests that an increased propensity to CSD could be a mechanism involved in the increased prevalence of migraine in women. Both oestrogen and progesterone have been reported to increase the frequency of CSD (Sachs et al. 2007).

Beyond the careful new migraine patient selection, the short- or long-term follow-up studies give hope for longitudinal remeasurements of the same patient group, to see further volume changes.

5.1.6 Limitations

The main limitation of this study is that migraine patients were not followed for at least one year to obtain precise data on the pain side. Another limitation is the cross-sectional nature of the study, which does not allow for evaluation of whether the observed volume changes are progressive during the disease course.

We did not adjust p-values for multiple comparisons. However, to avoid data dredging our investigation was restricted to a small number of brain regions predefined based on a priori hypothesis. The choice of thalamus and hippocampus was based on previous studies, and we have good reasons to believe that both could be involved in migraine. Adjusting p-values for multiple comparisons is still controversial and not always the right choice, especially in studies with a clearly defined primary hypothesis (Althouse 2016).

Thalamus segmentation was performed by Freesurfer 5.3. Having analyzed quite a few of our initial measurements with this version, we did not want to switch to another major version released in the meantime. Such an update is discouraged without repeating all the analyses already successfully completed by Freesurfer 5.3.

5.2 Volumetric Alteration of Brainstem in Female Migraineurs With and Without Aura

In the present study, we investigated a rather homogeneous migraine group to explore the brainstem volume alteration in female migraineurs as well as the potential effects of aura and other migraine characteristics on brainstem volume. Our results demonstrated that migraineurs had greater medulla volume compared to healthy controls, and also suggested the aura is not the primary cause of brainstem volume changes.

Previous imaging studies comparing the two subgroups of migraine (i.e., MwA and MwoA) were mostly conducted using perfusion or fMRI techniques (Datta et al. 2013; del Rio et al. 1999; Harita and Stroman 2017; Kreczmanski et al. 2019; Tessitore et al. 2015). An fMRI study indicated that there were no significant differences in the executive control network (commonly including prefrontal cortex, frontopolar cortex, anterior cingulate cortex and posterior parietal cortex) functional connectivity between MwA and MwoA groups (Tessitore et al. 2015). Interestingly, Datta and colleagues (2013) found higher BOLD fMRI response to visual stimulus within the occipital pole in MwA compared to MwoA, but resting cerebral blood flow of primary visual cortex was not different between these two groups. Additionally, during an interictal phase, Kreczmański et al. (2019) found BOLD fMRI activity differences in response to visual stimuli within the brainstem of MwA compared to MwoA. However, another perfusion-weighted imaging study indicated that during the aura there were no hemodynamic changes in the brainstem or other anatomical areas except the occipital cortex (del Rio et al. 1999). Based on the above-mentioned studies, we assume there are subtle functional differences in the brain between MwA and MwoA groups (especially in the occipital lobe and brainstem), which may be also associated with morphological changes. However, functional data obtained from brainstem are affected by technical challenges that can influence the quality of the results, which also limits its use in this region (Harita and Stroman 2017). In the present study, we did not find a significant difference in the volume of whole brainstem and its subfields between MwA and MwoA. Future studies combining morphological and functional measures are needed to further

investigate the brainstem and assess the potential correlation between these modalities. Especially, longitudinal studies could provide more information as to whether functional changes precede morphological alterations and whether they indicate findings in the same part of the brainstem.

Central sensitization may contribute to migraine due to atypical modulation of pain by the descending pain modulatory system (Mungoven et al. 2022; Schwedt et al. 2014; Yanes 2019). The medulla is the brain region with high density of areas involved in pain modulation, including the RVM, the nucleus of the solitary tract, dorsal reticular nucleus, ventral reticular nucleus and ventrolateral medulla (Pinto, et al. 2008). Excitatory midbrain nuclei and inhibitory medulla nuclei compose a circuit that controls cortical arousal levels (e.g., congnition and pain) (Thapaliya et al. 2023). A previous study (Chong et al. 2017b) found migraineurs had smaller midbrain volumes. Combined with our finding that migraine patients had greater medulla volumes, these structural changes might be the result of a disrupted regulation of the circulatory system. In addition, PAG dysfunction has also been implicated in different pain diseases including migraine (Maizels et al. 2012). fMRI studies found that PAG had significant functional connectivity with RVM (Kong et al. 2010). The PAG influences descending pain modulation primarily through its reciprocal connections with the RVM (Ossipov et al. 2014). Our results are consistent with previous studies indicating the evidence for a medullary brainstem mediator of pain (Kong et al. 2010; Maizels et al. 2012; Ossipov et al. 2014; Pinto et al. 2008). Pain modulation from the medulla is the only part of the endogenous pain control system from which facilitation of pain transmission can be produced along with antinociception (Tavares and Lima 2002). The brainstem nuclei in the RVM exclusively facilitate nociceptive spinal activity (Goksan et al. 2018). Edelmayer et al. (2009) conducted an animal experiment which demonstrated that injection of bupivacaine directly into the RVM could attenuate allodynia induced inflammatory mediators. Another recent animal experiment showed that injection of neuronal excitant into nucleus raphe magnus (NRM) and PAG selectively inhibited the responses of trigeminovascular second-order neurons to dura mater; meanwhile, injection of local anaesthetic into these two nuclei selectively potentiated the responses of these neurons to dura (Zagami et al. 2021). RVM is a key structure in descending

pain modulation, whereas the main function of the NRM is mostly pain mediator. These findings suggest that volume alteration may contribute to dysfunction of pain modulatory in medulla. Besides, we found that disease duration and medulla volume had a significant positive correlation, which supports a progressive increase of medulla volumes with long disease duration, but longitudinal studies are needed to prove in the future.

To date, there have been very limited morphological studies on brainstem of migraineurs, and the findings from these studies are variable (Chong et al. 2017b; Marciszewski et al. 2018; Petrusic et al. 2019; Qin et al. 2019). We did not find significant volume difference for the whole brainstem in migraine patients compared to healthy controls, which is in line with a previous study (Chong et al. 2017b; Qin et al. 2019). Nevertheless, Petrusic et al. (2019) found MwA had a greater whole brainstem volume compared to healthy controls, and increased volume changes were seen in pons and midbrain as well. However, this study only included MwA, which may partly contribute to the different results. Furthermore, previous studies were mostly conducted in migraine groups including both males and females (Chong et al. 2017b; Petrusic et al. 2019), while our study included only females. Maleki and colleagues (2012) have found stronger responses to noxious stimulation in SpV of the brainstem for female migraineurs relative to male migraineurs, which may be associated with sex difference in brainstem volume. The trigeminal nerve is thought to be involved in the pathophysiology of migraine, and SpV receives afferents from trigeminal nerve, which may lead to the volume changes of SpV in migraineurs. Two subsequent studies found reduced grey matter volume in SpV in migraine patients (Marciszewski et al. 2018; Qin et al. 2019), but unfortunately, neither of them investigated the effect of gender on their findings. SpV is a small nucleus/tract located in the lateral medulla of the brainstem. Despite the greater volume of medulla found in the present study, we could not determine if our results are conflicting, because we did not examine any subregions of the medulla. Future studies using a higher spatial resolution and specific image contrast – that makes possible the differentiation of medulla subregions – need to be performed to reliably assess morphological differences separately in each subregion. Additionally, these two studies used VBM approach, which could lead to different findings from

those obtained by FreeSurfer (Perlaki et al. 2018).

It is worth noting that none of above-mentioned studies investigated the impact of aura on brainstem volume. Although most of them excluded subjects with abnormal findings on T1 and T2 MR images, migraine aura could cause white matter microstructural differences (Szabo et al. 2018), which are invisible on routine clinical MR images but may still contribute to volume alteration of brain structures.

Beyond gender, aura and various other factors such as different image processing methods (VBM vs FreeSurfer), the number of study participants could also contribute to inconsistent findings from previous studies. In this context, it should be emphasized that our sample size is bigger and more homogeneous compared to most previous studies

Although our study revealed that aura has no obvious effect on brainstem volume changes, we did not obtain an fMRI scan on the same group to investigate the functional abnormalities. Moreover, the number of MwA is not sufficient for us to explore the impact of different types of migraine aura on brainstem volume, which needs to be considered for future studies. Additionally, because the number of MwoA with WMLs is relatively small, we did not investigate the effect of lesions on brainstem volume changes, which needs to be taken into consideration by future studies to better understand the conflicting findings from previous studies.

6. Summary

It has been demonstrated that migraine patients show different structural brain abnormalities, which include white matter lesions, volumetric changes in grey matter and white matter. Previously, we studied the possible influence of WMLs on hemispheric cortical thickness and volume in the same group of female migraineurs, but statistically significant correlations were not found.

In this thesis, we firstly tested the hypotheses whether WMLs and the migraine characteristics (disease duration, frequency, and aura) may affect the volume of both the thalamus and hippocampus in female migraine group, and then explored whether aura and other migraine characteristics may lead to volume changes of brainstem and its subfields for migraineurs without WMLs. For all of these brain structures, we also investigated the potential volume differences between the migraine and the healthy control groups.

The major findings of this research are the following: 1.) the volume of the right thalamus is decreased in migraineurs with WMLs, 2.) the volume of right hippocampal body and tail are smaller in migraineurs with aura or WMLs, 3.) the volumes of both the left and right hippocampal tail are smaller in patients with higher migraine frequency, 4.) the volume of medulla is bigger in migraineurs compared to healthy controls, 5.) aura has no significant impact on the volumetric change of brainstem and its subfields, 6.) the volume of medulla is bigger in migraine patients with longer disease duration.

These structural abnormalities are likely to be the consequence of recurrent migraine headache attacks. The patients need to follow a healthy lifestyle and avoid almost all migraine risk factors, and the doctors need to investigate co-morbidities if suspected and control the attacks with the most effective therapy.

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8. List of publications

8.1 Articles related to this thesis

He M, Kis-Jakab G, Komáromy H, Perlaki G, Orsi G, Bosnyák E, Rozgonyi R, John F, Trauninger A, Eklics K, Pfund Z. The volume of the thalamus and hippocampus in a right-handed female episodic migraine group. Frontiers in Neurology. 2023 Oct 19;14:1254628. (IF: 3.4)

He M, Kis-Jakab G, Komáromy H, Perlaki G, Orsi G, Bosnyák E, Rozgonyi R, John F, Trauninger A, Eklics K, Pfund Z. Volumetric alteration of brainstem in female migraineurs with and without aura. Clinical Neurology and Neurosurgery. 2023 Dec 19;236:108089. (IF: 1.9)

8.2 Article related to the project this thesis based on

Komáromy H, **He M**, Perlaki G, Orsi G, Nagy SA, Bosnyák E, Kamson Olayinka D, John F, Trauninger A, Pfund Z. Influence of hemispheric white matter lesions and migraine characteristics on cortical thickness and volume. The Journal of Headache and Pain. 2019 Jan 10;20(1):4.

8.3 Articles unrelated to this thesis

He M, Xu M, Cao Z. The advances of imaging research in pulmonary ground-glass nodules. International Journal of Medical Radiology, 2014,37(2): 123-126; 151

He M, Xu M, Yu Y, WangW. CT and MRI imaging diagnosis of pheochromocytoma. Journal of Medical Imaging, 2014, 24(7): 1259-1261

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8.4 Presentations unrelated to this thesis

He M. A classified study of the volume density characteristics in solitary solid pulmonary nodules. European Congress of Radiology, Vienna, Austria, 2018

He M, Gao X, Zhou K, Chen G, Dai H. CT diagnosis of pulmonary aspergillosis. The Thirteenth Session of Chinese Imaging of Integrated Traditional and Western Medicine annual meeting, Wuyishan, China, 2014.

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He M, Xu M, Yang G. CT appearance of primary retroperitoneal tumors. The Thirteenth Session of Chinese Imaging of Integrated Traditional and Western Medicine annual meeting, Wuyishan, China, 2014.

He M, Zhou C, Xiang P, Pu Y, Liu Y. CT and MRI features and diagnosis of myelolipoma. The Thirteenth Session of Chinese Imaging of Integrated Traditional and Western Medicine annual meeting, Wuyishan, China, 2014.

9. Acknowledgements

First of all, I would like to thank Prof. Tamás Dóczi for guiding me to conduct research work in the field of clinical neuroscience, and giving support for my study.

I am deeply grateful to my supervisor, Prof. Zoltán Pfund, to let me to join migraine research team, and guided me throughout the research work from title's selection to finding the results. And I also want to express my sincere gratitude to my co-supervisor Dr. Gábor Perlaki for invaluable guide, continuous support, and patience during my PhD study. Words cannot express my gratitude to you for the kindness and support I have received.

I need to thank Dr. Gergely Orsi and Dr. Szilvia Anett Nagy. Their immense knowledge and plentiful experience have encouraged me in all the time of my academic research in Pécs. I want to thank Gréta Kis-Jakab for help and advice.

I would like to extend my sincere thanks to Kinga Stankovics and her family. The friendship between your family and me let me feel warm and belonged to stay in the place where is quite far away from my hometown.

In the end, I am tremendously grateful for the unconditional love and support from my parents and brother. You all are my strong backing.

frontiers | Frontiers in Neurology

TYPE Original Research
PUBLISHED 19 October 2023
DOI 10.3389/fneur.2023.1254628

OPEN ACCESS

EDITED BY
Raffaele Ornello,
University of L'Aquila, Italy

REVIEWED BY
Ana Starcevic,
University of Belgrade, Serbia
Csaba Juhasz,
Wayne State University, United States
David Olayinka Kamson,
Johns Hopkins Medicine, United States
Mansoureh Togha,
Tehran University of Medical Sciences, Iran

*CORRESPONDENCE
Zoltán Pfund
✉ pfund.zoltan@pte.hu

RECEIVED 07 July 2023
ACCEPTED 25 September 2023
PUBLISHED 19 October 2023

CITATION
He M, Kis-Jakab G, Komáromy H, Perlaki G, Orsi G, Bosnyák E, Rozgonyi R, John F, Trauninger A, Eklics K and Pfund Z (2023) The volume of the thalamus and hippocampus in a right-handed female episodic migraine group. *Front. Neurol.* 14:1254628. doi: 10.3389/fneur.2023.1254628

The volume of the thalamus and hippocampus in a right-handed female episodic migraine group

Mingchen He[1], Gréta Kis-Jakab[2,3,4], Hedvig Komáromy[1], Gábor Perlaki[1,2,3,4], Gergely Orsi[1,2,3,4], Edit Bosnyák[4], Renáta Rozgonyi[4], Flóra John[4], Anita Trauninger[4], Kata Eklics[5] and Zoltán Pfund[4]*

[1]Pécs Diagnostic Center, Pécs, Hungary, [2]The Hungarian Research Network-Pécsi Tudományegyetem, Clinical Neuroscience Magnetic Resonance Research Group, Pécs, Hungary, [3]Department of Neurosurgery, Medical School, University of Pécs, Pécs, Hungary, [4]Department of Neurology, Medical School, University of Pécs, Pécs, Hungary, [5]Department of Languages for Biomedical Purposes and Communication, University of Pécs, Pécs, Hungary

Background/aim: Migraine is a disabling headache with clinical and radiological complications. The aim of this study was to investigate the volume of the thalamus and hippocampus in migraineurs, the role of white matter lesions (WMLs), and the migraine characteristics in volume changes.

Methods: Brain MRIs of 161 right-handed female episodic migraine patients and 40 right-handed, age-related, healthy women were performed. Left and right thalamus segmentation was performed on the 3D MPRAGE images using the Freesurfer 5.3 image analysis suite. Hippocampal subfield segmentation was based on a novel statistical atlas built primarily upon ultra-high-resolution *ex vivo* MRI data.

Results: The left hippocampus had a smaller and the left thalamus had a larger total volume than the right one in both the control ($p < 0.001$) and migraine groups ($p < 0.001$). Patients with white matter lesions (L+) showed smaller right thalamus and right hippocampal tail volumes than patients without lesions (L−) ($p = 0.002$ and $p = 0.015$, respectively) and controls ($p = 0.039$ and $p = 0.025$, respectively). For the right hippocampal body, we found significantly smaller volume in L+ patients when compared to L− patients ($p = 0.018$) and a similar trend when compared to the control group ($p = 0.064$). Patients without aura (A−) showed a larger right hippocampus ($p = 0.029$), right hippocampal body ($p = 0.012$), and tail volumes ($p = 0.011$) than patients with aura (A+). Inverse correlations were found between attack frequency and the volumes of the left and right hippocampal tails ($p = 0.018$ and $p = 0.008$, respectively).

Conclusion: These findings indicate that WMLs may influence the volume of the right thalamus and hippocampus, while migraine aura and attack frequency may lead to volume changes in different parts of the hippocampi in migraine patients. These data support the necessity of effective migraine management to limit subcortical volume loss in migraineurs.

KEYWORDS

episodic migraine, white matter lesions, migraine headache characteristics, volume of thalamus and hippocampus, right-handed female migraineurs

Introduction

Migraine is a painful, returning headache disease that lasts for decades (1). Migraine is more prevalent in women than men due to hormonal differences starting in puberty (2–4). In addition, the migraine characteristics of men and women are different. Women report a longer attack duration, increased risk of headache recurrence, higher intensity of headaches, more frequent nausea, phonophobia, and photophobia, greater disability, and a longer time required for recovery (2–4).

Magnetic resonance imaging (MRI) is a useful diagnostic tool in migraine and shows migraine-related structural complications, e.g., hemispheric white matter lesions (WMLs) as well as cortical and subcortical volume changes of different structures (5, 6). The WMLs are clinically mute, mostly progressive microvascular tissue damages, which may influence the intrahemispheric size and volume impairment (5). Previously, we studied the possible influence of WMLs on hemispheric cortical thickness and volume in 161 right-handed female migraineurs, but statistically significant correlations were not found (6).

In healthy adults, the total brain volume was reported to be larger from birth in men than in women by ∼11%. This size difference accounts for other reproducible findings: higher white/gray matter ratio, intra- vs. interhemispheric connectivity, and regional cortical and subcortical volumes in men. When structural and lateralization differences are present independent of size, sex/gender explains only ∼1% of the total variance (7). Furthermore, there are several documented structural and functional brain differences between men and women, but it is hard to decide which of them is sex hormone-related (8).

The trigeminovascular system is a pain-transmission link between the vascular (dural and cortical) and neuronal (brainstem and thalamus) regions (9). The thalamus is a relay station; thereby, it has high (density) connectivity to many brain structures. It holds an important position in allodynia, central sensitization, and photophobia in migraine (10).

The hippocampus is involved in memory consolidation, spatial navigation, and pain-related stress response (11), as well as in pain processing, pain-related attention, and anxiety (12). Stronger hippocampal-cortico-limbic connectivity in migraineurs is associated with allodynia (11).

Based on the current literature, both structures are functionally impaired in migraine; thereby, we hypothesized that the hemispheric tissue damage (WMLs) and the migraine characteristics (disease duration, frequency, and aura) may negatively affect the volume of both the thalamus and hippocampus.

To test our hypothesis, we reinvestigated our abovementioned migraine headache patients' brain MRI studies (6) to measure the volume of the thalami and hippocampi and to evaluate the potential role of WMLs and the migraine characteristics in volume abnormalities.

Abbreviations: ANCOVA, analysis of covariance; CSD, cortical spreading depression; FLAIR, fluid-attenuated inversion recovery; ICV, intracranial volume; 3D MPRAGE, three-dimensional magnetization-prepared rapid gradient-echo; MRI, magnetic resonance imaging; SPSS, statistical package for the social sciences; ROS, reactive oxygen species; SD, spreading depression; WMLs, white matter lesions.

Patients and methods

Subjects

A total of 161 women with episodic migraine (mean age 39.3 ± 12.5, range 18–73 years; disease duration 15.6 ± 11.9, range 1–57 years; attack frequency/month 5.6 ± 4.5, range 0.2–20.0; 63 with aura; 52 with white matter lesions; Table 1) were prospectively enrolled in this study. As controls, 40 age-matched healthy female subjects were included (mean age 38.3 ± 10.0, range 19–66 years). Migraineurs had no other types of headaches. None of the included migraine patients' headaches or auras were unilaterally side-locked in nature, but the headaches showed right hemisphere dominance in 68.3% (right-sided or bilateral with right > left pain intensity). An MRI was performed during a headache-free period for each patient; the minimum headache-free period was 1 day after the postdrome headache phase. All migraineurs and controls reported right-handedness. All subjects were strictly investigated for medical comorbidities (e.g., past and current medical history, physical examination, routine and autoimmune blood tests, cardiovascular monitoring, BMI, sleep disease, hypertension, gynecological disease, thyroid gland, and cognitive disease), and only those were enrolled in this study who lacked any medical diseases. Brain MRI studies of healthy participants did not show any structural abnormalities.

MRI acquisition

All subjects were scanned on the same 3T MRI scanner (Magnetom TIM Trio, Siemens AG, Erlangen, Germany) using a 12-channel head matrix coil. A whole-brain T1-weighted three-dimensional axial magnetization-prepared rapid gradient-echo (3D MPRAGE) sequence was acquired using the following parameters: TR/TI/TE = 1,900/900/3.4 ms, BW = 179 Hz/px, flip angle = 9°, FOV = 210 × 240 mm^2, matrix size = 224 × 256, slice thickness = 0.94 mm, 176 axial slices, 0.94 × 0.94 × 0.94 mm^3 isotropic voxels. Beyond the routine T1- and T2-weighted measurements, the scanning protocol also included fluid-attenuated inversion recovery (FLAIR) imaging (TR/TI/TE = 13,200/2,600/100 ms, BW = 401 Hz/px; echo trains = 14, FOV = 186 × 220 mm^2, matrix size = 162 × 192, slice thickness = 1.5 mm, 100 axial slices).

MR image analysis

Left and right thalamus segmentation was performed on the high-resolution three-dimensional 3D MPRAGE images using the Freesurfer 5.3 image analysis suite (https://surfer.nmr.mgh.harvard.edu/fswiki). The technical details were described previously (13). Quality control was performed throughout the automatic processing stream. When the reconstruction was inaccurate, error correction was performed based on the recommended workflow (http://surfer.nmr.mgh.harvard.

TABLE 1 Demographic and clinical data of migraine patients and healthy controls.

	Lesion+ ($n = 52$)	Lesion- ($n = 109$)	Controls ($n = 40$)	Differences (p-value)		
				L+ vs. L–	L+ vs. C	L– vs. C
Age (years)	44.6 ± 13.1 (20–72)	36.7 ± 11.4 (18–73)	38.3 ± 10.0 (19–66)	<0.001[a]	0.018[a]	0.422[a]
Disease duration (years)	19.8 ± 12.9 (1–57)	13.7 ± 10.9 (1–43)	–	0.003[a]	–	–
Migraine attack frequency/month	5.4 ± 4.2 (0.2–17.0)	5.7 ± 4.6 (0.5–20.0)	–	0.807[a]	–	–
Patients with aura	31 (59.6 %)	32 (29.4 %)	–	<0.001[b]	–	–

Lesion+, migraineurs with lesions; Lesion–, migraineurs without lesions. Values are given as mean ± standard deviation (minimum–maximum).
[a]Mann-Whitney U-test (two-sided exact p-value).
[b]Fisher's exact test (two-sided exact p-value).

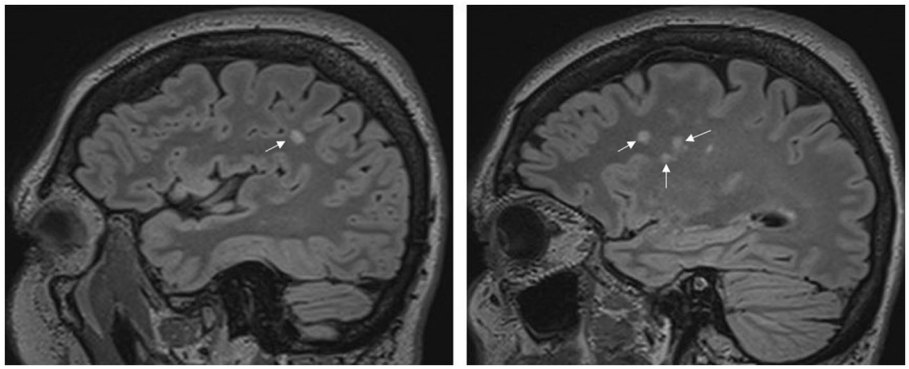

FIGURE 1
Migraine patient with intrahemispheric white matter lesions. The sagittal fluid-attenuated inversion recovery (FLAIR) images belong to one patient and show high signal intensities in the white matter. The arrows show the lesions.

edu/fswiki/RecommendedReconstruction). The left and right hippocampus and their subfields (head, body, and tail) were segmented using the development version of FreeSurfer on 31 August 2017 (https://surfer.nmr.mgh.harvard.edu/fswiki/DownloadAndInstall). This new hippocampal subfield segmentation method is based on a novel statistical atlas built primarily upon ultra-high-resolution (~0.1 mm isotropic) *ex vivo* MRI data. This *ex vivo* atlas outperforms the *in vivo* counterpart that was distributed with Freesurfer 5.3 and yields subregion volumes that agree better with those from histological studies. The technical details of this new method were described earlier (14).

Analysis of white matter lesions

WMLs were considered if visible as hyperintensity on T2-weighted and FLAIR MRI but without a low-signal intensity lesion on T1-weighted MRI and larger than 3 mm, appearing in at least two consecutive slices (Figure 1). Supratentorial WMLs were delineated manually on the FLAIR images using the 3D Slicer software (http://www.slicer.org, Version 4.6.2, Figure 2). Total and lobar hemispheric WML numbers/volumes were calculated for all L+ subjects. The borders of the lobes were defined as previously described (6).

Statistical analysis

Statistical analyses were performed using the SPSS 20.0 software (IBM Corp., Armonk, NY, United States).

Differences in age between the whole migraine group and healthy controls were assessed using the Mann-Whitney U-test. Age differences among migraine subgroups (L+ and L–) and the healthy control group were assessed using the Kruskal–Wallis test followed by the Mann-Whitney U-test. The age difference between migraine patients without aura (A–) and those with aura (A+) was assessed using the Mann-Whitney U-test.

A Wilcoxon signed-rank test was used to identify whether there were statistically significant differences between the total volume of the left and right hippocampus, as well as between the left and right thalamus, separately in the healthy control group and the migraine group. Volume differences between the healthy control group and the migraine group in the left and right hippocampus, as well as the thalamus, were assessed by ANCOVA using age and total intracranial volume (ICV) as covariates.

Differences in continuous migraine-related variables (i.e., disease duration, migraine attack frequency) between migraine subgroups (L+ and L–) were compared using the Mann–Whitney U-test, while differences in the rate of aura (A+ or A–) between the same subgroups were assessed using Fisher's exact test. Within

the migraine group with lesions (L+), a Wilcoxon signed-rank test was used to test for differences between left and right hemispheres in total and lobar hemispheric WML numbers/volumes. These differences were not tested for the temporal and occipital lobes because lesions were rare in these lobes, with a median lesion number of 0.

Differences in the thalamus, hippocampal, and hippocampal subfield volumes among the three groups (L+, L–, and control) were assessed using ANCOVA with age and ICV as covariates. In the case of significance, the least significant difference *post-hoc* analyses were conducted for pairwise comparison of the three groups.

Within the whole migraine group, the same volumes were compared between patients with aura (A+) and without aura (A–) using ANCOVA with age and ICV as covariates. The possible effects of other migraine characteristics (i.e., disease duration and migraine attack frequency) on the examined volumes were tested using stepwise multiple linear regression analyses. In these models, age, ICV, lesion, and aura group variables were also included as possible predictors.

Within the migraine group with lesions (L+), we assessed the potential relationships between left and right hemispheric brain structure volumes and the total and lobar lesion numbers/volumes in the corresponding hemisphere. Since the lesion number/volume data were not normally distributed (right-skewed with several extreme values), to perform powerful statistical analyses, the effects of lesion numbers/volumes were assessed by creating binarized subgroup variables based on the median split of them, e.g., the subgroup with a low number/volume (below or equal to the median) vs. the subgroup with a high number/volume of left hemispheric frontal lobe lesions. The effects of these subgroup variables on the examined volumes were tested using stepwise multiple linear regression analyses, including all these subgroup variables, age, and ICV as possible predictors. Since lesions were rare in the temporal and occipital lobes with a median lesion number of 0, the effects of lesion number/volume in these lobes were not assessed. Results were considered significant at $p \leq 0.05$.

Results

There was no significant difference in age between the whole migraine group and the control group ($p = 0.736$). The Kruskal–Wallis test revealed significant age differences among the migraine subgroups (L+ and L–) and controls ($p = 0.001$). *Post-hoc* testing indicated that the L+ subgroup was significantly older than the other two groups (Table 1). There was no significant difference in age between patients without aura (A–) and with aura (A+) ($p = 0.816$, two-sided exact p-value).

The Wilcoxon signed-rank test showed that the left hippocampus had a smaller total volume when compared with the right hippocampus in both the healthy control group (3,353 ± 252 mm^3 vs. 3,517 ± 289 mm^3, $Z = -4.968$, $p < 0.001$) and the migraine group (3,379 ± 293 mm^3 vs. 3,494 ± 317 mm^3, $Z = -8.653$, $p < 0.001$). On the other hand, the left thalamus had a bigger volume when compared with the right thalamus in both the healthy control group (7,708 ± 747 mm^3 vs. 6,984 ± 562 mm^3; $Z = -5.275$, $p < 0.001$) and the migraine group (7,675 ± 817 mm^3 vs. 6,951 ± 689 mm^3, $Z = -10.835$, $p < 0.001$). There were no significant differences between the healthy control group and the whole migraine group in the total volume of the left hippocampus ($p = 0.703$), right hippocampus ($p = 0.436$; one outlier patient was excluded based on standardized residuals > 3), left thalamus ($p = 0.801$; one outlier patient was excluded based on standardized residuals < −3), and right thalamus ($p = 0.726$).

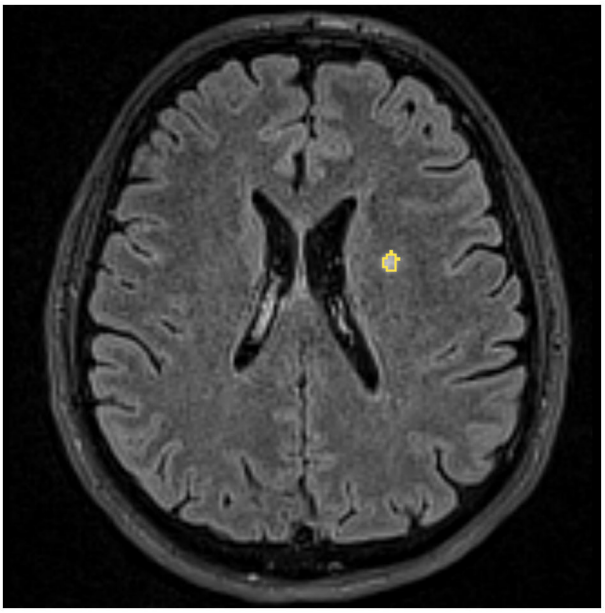

FIGURE 2
Delineation of a white matter lesion in a female migraineur. An example of an axial FLAIR image used to assess white matter lesion is shown in the background. Yellow indicates the outline of a manually delineated white matter lesion.

Disease duration and the rate of aura were significantly higher in L+ patients, while migraine attack frequency was not significantly different between the L+ and L– subgroups (Table 1). Results for the number and volumes of supratentorial white matter lesions in L+ patients in different hemispheric lobes and the total left and right hemispheres are presented in Table 2. The Wilcoxon signed-rank test indicated that there were no differences between the left and right hemispheres in total ($p = 0.341$), frontal ($p = 0.084$), and parietal ($p = 0.264$) WML numbers, and no differences were found for the total ($p = 0.448$) and frontal ($p = 0.226$) lesion volumes. However, the lesion volume was bigger in the left compared to the right-hemispheric parietal lobe ($Z = -2.097$, $p = 0.036$).

After correcting for the confounding effects of age and ICV, the patients with lesions (L+) showed significantly smaller right thalamus and right hippocampal tail volumes than patients without lesions (L–) and controls (Table 3). For the right hippocampal body, the ANCOVA showed a strong trend ($p = 0.051$); therefore, we performed the *post-hoc* tests, which indicated a significantly smaller volume in L+ patients when compared to L– patients and a trend when compared to the control group (Table 3).

Focusing on the migraine group, patients without aura (A–) showed a larger right hippocampus, right hippocampal body, and tail than patients with aura (A+) (Table 4).

TABLE 2 Number and volumes of supratentorial white matter lesions in the Lesion+ migraine subgroup.

Location	Left hemisphere		Right hemisphere	
	Lesion number	Lesion volume (mm^3)	Lesion number	Lesion volume (mm^3)
Total	5 (0–41)	171 (0–4,343)	5 (0–39)	153 (0–4,632)
	8	355	8.75	354
Frontal lobe	3 (0–24)	68 (0–1,542)	3 (0–26)	92 (0–3,283)
	5	231	6	180
Parietal lobe	1 (0–11)	31 (0–2,558)	0.5 (0–11)	6 (0–1,749)
	4	167	3	115
Temporal lobe	0 (0–4)	0 (0–274)	0 (0–6)	0 (0–658)
	1	27	1	21
Occipital lobe	0 (0–2)	0 (0–183)	0 (0–2)	0 (0–374)
	0	0	0	0

Lesion+, migraine patients with lesions. Values are given as median (minimum–maximum) and interquartile range, separately for each hemispheric lobe and for the total left and right hemispheres.

Within the whole migraine group, neither the disease duration nor migraine attack frequency was selected by stepwise linear regression as significant predictors of the examined structural volumes, except for the volumes of hippocampal tails. Using multiple linear regression, including the volume of the left hippocampal tail as the dependent variable and migraine attack frequency, age, and ICV as independent variables, we found a significant negative correlation between attack frequency and the volume of the left hippocampal tail ($p = 0.018$, $t = -2.385$, Figure 3A). Based on our results reported in Tables 3, 4, for the right hippocampal tail, we also included the lesion (L– vs. L+) and aura (A– vs. A+) in addition to migraine attack frequency, age, and ICV as independent variables in the multiple linear regression model. We found a negative correlation of attack frequency with the right hippocampal tail volume ($p = 0.008$, $t = -2.688$, Figure 3B).

In the L+ migraine group, none of the binarized total or lobar hemispheric lesion number/volume variables were selected by stepwise linear regression as significant predictors of the examined structural volumes.

Discussion

In this rather homogenous patient population, we investigated 161 right-handed episodic migraineurs. To eliminate the confounding effect of sex-related differences in headache characteristics and brain size, only women were enrolled in the study. The total volumes of the left or right thalami and hippocampi were not different between migraineurs and controls. The volume of the right thalamus was smaller than the left one within the migraine and control groups. Patients with WMLs showed a smaller right thalamus than in lesion-negative migraineurs. The hippocampi were smaller on the left side in both groups. The hippocampus subgroup analysis of migraine patients and the subfield volume values of all three groups showed statistically significant differences. The white matter lesions, the presence of aura, and the attack frequency appeared to be risk factors for smaller volumes. It is also possible that the dominant unilateral headache side with more attacks on the right side has a role in the volume differences of the thalami. Since the patients' memory was only reliable for the last month, we did not use these data for statistical analysis.

Thalamic and hippocampal volume asymmetries in healthy subjects

In our right-handed healthy female control group, significant volume differences were detected between the thalami (R < L) and hippocampi (R > L) of the two hemispheric sides. Since in the cited morphometric studies (15–17), the participants were not separated by sex, and handedness was not documented in one study, these findings are just partly comparable with our data. However, handedness may influence the hippocampus volume on the same side.

Manual dominance in migraine

In a large retrospective study, the authors collected data from 546 patients with migraine aged between 16 and 65 years, reporting manual dominance according to the Edinburgh test. They included 466 right-handed and 80 left-handed subjects with migraine and registered 4,215 unilateral painful attacks. It was concluded that manual dominance influences the side of pain lateralization in migraine (18). Considering our 1-month-long database, the right-handed data are congruent.

The volume of the thalamus in migraine

In a study of 35 patients with migraine without aura and 40 healthy controls, the authors found thalamic nuclei with significantly increased and decreased volumes. The authors

TABLE 3 Group differences in the volumes of examined structures among lesion-related migraine subgroups and controls.

	Groups			Statistics				
	L+	L–	Control	ANCOVA test		LSD *post-hoc* test		
				F (df1, df2)	*P*	L+ vs. L–	L+ vs. C	L– vs. C
L thalamus	7,548 (914)	7,758 (727)	7,708 (747)	1.639 (2,194)	0.197	–	–	–
R thalamus	6,698 (689)	7,072 (659)	6,984 (562)	**5.092 (2,195)**	**0.007**	**0.002**	**0.039**	0.560
L hippocampus	3,340 (345)	3,397 (265)	3,353 (252)	0.735 (2,195)	0.481	–	–	–
R hippocampus	3,434 (364)	3,522 (289)	3,517 (289)	1.722 (2,195)	0.181	–	–	–
L hippocampal head	1,689 (194)	1,709 (157)	1,685 (124)	0.311 (2,195)	0.733	–	–	–
R hippocampal head	1,742 (215)	1,756 (156)	1,762 (160)	0.288 (2,194)	0.750	–	–	–
				0.438[a] (2,192)	0.646[a]			
L hippocampal body	1,150 (102)	1,158 (84)	1,140 (80)	0.903 (2,189)	0.407	–	–	–
R hippocampal body	1,153 (115)	1,191 (94)	1,185 (103)	3.022 (2,194)	0.051	**0.018**	0.064	0.928
L hippocampal tail	504 (70)	524 (62)	518 (67)	1.672 (2,194)	0.191	–	–	–
				1.776[b] (2,193)	0.172[b]			
R hippocampal tail	539 (71)	567 (67)	570 (56)	**3.589 (2,195)**	**0.029**	**0.015**	**0.025**	0.766

L+, migraine patients with lesions; L–, migraine patients without lesions; ICV, total intracranial volume; L, left; R, right; LSD, least significant difference.

Subjects were excluded from the analysis if the corresponding standardized residuals from the ANCOVA model were below −3 or above 3. A maximum of three subjects had to be excluded from either group. Volumes are presented as uncorrected mean (standard deviation) in mm^3 for subjects included in the ANCOVA statistics. For *post-hoc* tests, *P*-values are given.

[a]Statistical values are also presented for the group effect at mean ICV when including the significant Group*ICV interaction term in the model.

[b]Statistical values are also presented for the group effect when including the significant Age*ICV interaction term in the model.

Bold values denote statistical significance.

10.3389/fneur.2023.1254628

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TABLE 4 Group differences in the volumes of examined structures between aura-related migraine subgroups.

	Groups		ANCOVA test	
	Aura+	Aura-	*F* (df1, df2)	*P*
L thalamus	7,655 (942)	7,688 (729)	0.295 (1,157)	0.588
R thalamus	6,966 (765)	6,941 (640)	0.003 (1,157)	0.955
L hippocampus	3,339 (328)	3,404 (267)	3.004 (1,157)	0.085
R hippocampus	3,439 (346)	3,529 (293)	**4.849 (1,157)**	**0.029**
L hippocampal head	1,685 (192)	1,714 (153)	1.947 (1,157)	0.165
R hippocampal head	1,739 (208)	1,759 (154)	1.115 (1,156)	0.293
			1.141[a] (1,155)	0.287[a]
L hippocampal body	1,143 (106)	1,164 (96)	2.615 (1,156)	0.108
R hippocampal body	1,158 (107)	1,192 (98)	**6.504 (1,156)**	**0.012**
L hippocampal tail	512 (67)	524 (66)	1.654 (1,157)	0.200
R hippocampal tail	542 (63)	569 (71)	**6.669 (1,157)**	**0.011**
			6.998[b] (1,156)	**0.009[b]**

Aura+, migraine patients with aura; Aura-, migraine patients without aura; ICV, total intracranial volume; L, left; R, right.
Subjects were excluded from the analysis if the corresponding standardized residuals from the ANCOVA model were below −3 or above 3.
Maximum one subject had to be excluded from the Aura- group. Volumes are presented as uncorrected mean (standard deviation) in mm^3 for subjects included in the ANCOVA statistics.
[a]Statistical values are also presented for the group effect when including the significant Age*ICV interaction term in the model.
[b]Statistical values are also presented for the group effect at mean ICV when including the significant Group*ICV interaction term in the model.
Bold values denote statistical significance.

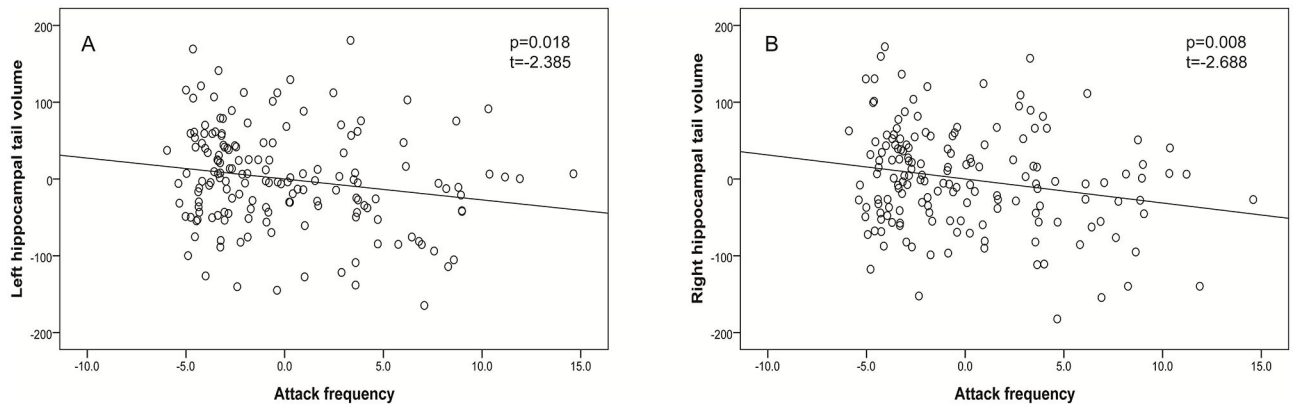

FIGURE 3
Partial regression plots demonstrating that more frequent migraine attacks were associated with decreased volumes of the left hippocampal tail (after controlling for the effects of age and ICV, **A**) and the right hippocampal tail (after controlling for the effects of age, ICV, lesion, and aura, **B**).

concluded that the result might contribute to the underlying pathogenesis of migraine (19).

In another study, significant volume reductions of three thalamic nuclei were observed in migraineurs: the central nuclear complex, the anterior nucleus, and the lateral dorsal nucleus (20). The thalamic nuclei with reduced volumes were connected to the limbic system.

In measurements of thalamic subregions in 77 migraineurs with episodic migraine and 30 controls, selective functional hypoconnectivity in the thalamic subregions was detected (21). The data provided neuroimaging evidence of thalamocortical pathway dysfunction in episodic migraines, including emotion and personality traits in migraineurs.

In a recent thalamic study, the volume was investigated by analyzing MRI data obtained from a large study, which specifically included women migraineurs with aura, unrelated migraine-free matched controls, and migraine aura-free co-twins. None of the analyses showed any between-group differences in the volume of the thalamus or of individual thalamic nuclei. These results indicated that the pathophysiology of migraine with aura did not involve alteration of thalamic volumes (22). According to our findings, thalamic volume abnormalities were not detected in migraine aura patients in the latter publication, but we found WML-associated smaller volume.

The volume of the hippocampus in migraine

In migraine patients, after a long follow-up for 2 years, the right hippocampus volume was positively associated with a good migraine outcome after adjustment of headache frequency (OR 4.72, $p = 0.024$) (23).

In a review of hippocampal volume, a longitudinal study discovered decreased volume in newly diagnosed migraine patients after 1 year (24). A cross-sectional study also suggested an adaptive increase in volume at low headache frequency and a maladaptive decrease in volume at higher headache frequency (25). These results were interpreted as either initial adaptive plasticity of the hippocampus or a larger hippocampus (i.e., a pre-existing condition) in migraineurs that then decreases with increased attack frequency (26). Evidence for the first interpretation has come from animal models of neurogenesis in the structure that is known to persist into adulthood. Stress (e.g., migraine) may mediate adaptive structural plasticity through the remodeling of dendrites and synapses (27). With repeated stress involved in migraine attacks, including pain, glucocorticoids, cortical spreading depression, and gonadal hormones, elevated and prolonged levels of excitatory amino acids are likely released (28, 29). Moreover, the aging rat hippocampus displays elevated and prolonged levels of excitatory amino acids released during acute stress. It is probable that structural plasticity in response to repeated stress starts as an adaptive and protective response but ends up as damage if the imbalance in the regulation of the key mediators is not resolved. It is likely that morphological rearrangements in the hippocampus impair memory functions, and it is conceivable that these may also have a role in chronic pain perception (30). In another study, the authors concluded that migraineurs had smaller hippocampal volume and stronger hippocampal-cortico-limbic connectivity compared to healthy subjects. Hippocampal volumes and measures of hippocampal volume connectivity with other cortico-limbic network regions are associated with symptoms of allodynia (11). These studies show hippocampal volume changes and support the role of migraine frequency in volume abnormalities. Since we also found a hippocampal tail volume association with migraine frequency, these findings are consistent with our results.

WMLs and migraine characteristics

The thalamic and hippocampal volume changes are likely the consequence of recurrent migraine headaches and accumulate with migraine years (5, 31). The WMLs are worsening with longer migraine disease duration, but they can be found at any age of migraineurs (5). A previous MRI study of supratentorial white matter hyperintensities in migraine patients demonstrated tissue damage with axonal loss, decreased glial cell density with impaired energy metabolism, enlarged extracellular space with an increased extracellular water fraction, and decreased blood flow and volume (31). In addition, reactive oxygen species (ROS) are the contributors to oxidative stress, which can cause vascular endothelial dysfunction in migraines and may lead to tissue damage in WMLs (32).

Both the thalamus and hippocampus are involved in migraine bout-related pain processing, which thereby causes stress. These include headache intensity, frequency, and the dominant hemisphere attack. The pain may have a role in the volume changes, especially in the hippocampi.

Migraine aura is usually unilateral and visual, but it can also be sensory, motor, brainstem, and retinal or cause speech/language disturbance. The cortical spreading depression (CSD) is a slowly propagated wave of depolarization of neurons and glial cells, followed by a subsequent sustained suppression of spontaneous neuronal activity, accompanied by complex and variable changes in vascular caliber, blood flow, and energy metabolism. Although spreading depression (SD) has been most extensively studied in the cortex, the phenomenon may occur in all neural tissues, including the hippocampus, cerebellum, and retina, among other regions (33, 34). Growing evidence suggests that an increased propensity to CSD could be a mechanism involved in the increased prevalence of migraine in women. Both estrogen and progesterone have been reported to increase the frequency of CSD (35).

Beyond the careful new migraine patient selection, the short- or long-term follow-up studies give hope for longitudinal remeasurements of the same patient group, to see further volume changes.

Limitations

The main limitation of this study is that migraine patients were not followed for at least 1 year to obtain precise data on the pain side. Another limitation is the cross-sectional nature of the study, which does not allow for an evaluation of whether the observed volume changes are progressive during the disease course.

We did not adjust *p*-values for multiple comparisons. However, to avoid data dredging, our investigation was restricted to a small number of brain regions predefined based on *a priori* hypothesis. The choice of thalamus and hippocampus was based on previous studies, and we have good reasons to believe that both could be involved in migraine. Adjusting *p*-values for multiple comparisons is still controversial and not always the right choice, especially in studies with a clearly defined primary hypothesis (36).

Thalamus segmentation was performed by Freesurfer 5.3. Having analyzed quite a few of our initial measurements with this version, we did not want to switch to another major version released in the meantime. Such an update is discouraged without repeating all the analyses already completed by Freesurfer 5.3.

Conclusion

The major findings of this study are the following: (1) the volume of the right thalamus is decreased in the white matter lesion migraine subgroup, (2) the volume of the right hippocampus is smaller in migraineurs with aura and WMLs, and (3) the volume of both hippocampus tails is smaller in patients with a higher migraine frequency. These structural abnormalities are likely to be the consequence of recurrent migraine headaches and their negative impact on the traditional migraine characteristics and progressive

nature of WMLs. These processes may negatively influence the function of the thalamus and hippocampus in the human brain. These data further support the importance of effective migraine management; briefly, the patients need to follow a healthy lifestyle and avoid almost all migraine risk factors, and the doctors need to investigate co-morbidities if suspected and control the attacks with the most effective therapy.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by Regional Research Ethics Committee of the Medical Center, Pécs. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

MH: Conceptualization, Formal analysis, Methodology, Writing—review and editing. GK-J: Data curation, Investigation, Writing—review and editing. HK: Formal analysis, Writing—review and editing. GP: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing—original draft, Writing—review and editing. GO: Formal analysis, Writing—review and editing. EB: Data curation, Investigation, Writing—review and editing. RR: Data curation, Investigation, Writing—review and editing. FJ: Data curation, Investigation, Writing—review and editing. AT: Data curation, Investigation, Writing—review and editing. KE: Writing—original draft, Writing—review and editing. ZP: Conceptualization, Data curation, Investigation, Methodology, Writing—original draft, Writing—review and editing.

Funding

This study was supported by grants from EEA/Norwegian Financial Mechanism HU 0114—Save what can be saved—applied neurological research using high-field magnetic resonance imaging, by TÁMOP-4.2.1/B, EFOP-3.6.2-16-2017-00008. The role of neuroinflammation in neurodegeneration: from molecules to clinics, TKP2021-EGA-16 and TKP-2021-EGA-13. Project Nos. TKP2021-EGA-16 and TKP-2021-EGA-13 have been implemented with the support provided by the National Research, Development and Innovation Fund of Hungary, financed under the TKP2021-EGA funding scheme. This study was also supported by the Hungarian Brain Research Program 2 and 3 (2017-1.2.1-NKP-2017-00002 and NAP 3.0) and the Translational Neuroscience National Laboratory (RRF-2.3.1-21-2022-00011). ZP was funded by PTE ÁOK-KA-2017-23 and EFOP-3.6.3-VEKOP_00009.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Clinical Neurology and Neurosurgery 236 (2024) 108089

Contents lists available at ScienceDirect

Clinical Neurology and Neurosurgery

journal homepage: www.elsevier.com/locate/clineuro

Volumetric alteration of brainstem in female migraineurs with and without aura

Mingchen He [a], Gréta Kis-Jakab [b,c,d], Hedvig Komáromy [a], Gábor Perlaki [a,b,c,d], Gergely Orsi [a,b,c,d], Edit Bosnyák [d], Renáta Rozgonyi [d], Flóra John [d], Anita Trauninger [d], Kata Eklics [e], Zoltán Pfund [d,*]

[a] Pécs Diagnostic Center, Pécs, Hungary
[b] HUN-REN-PTE Clinical Neuroscience MR Research Group, Pécs, Hungary
[c] Department of Neurosurgery, Medical School, University of Pécs, Pécs, Hungary
[d] Department of Neurology, Medical School, University of Pécs, Pécs, Hungary
[e] Department of Languages for Biomedical Purposes and Communication, University of Pécs, Pécs, Hungary

ARTICLE INFO

Keywords:
Magnetic resonance imaging
Episodic migraine
Aura
Brainstem volume
Morphometry

ABSTRACT

Background and Aim: Brainstem descending modulatory circuits have been postulated to be involved in migraine. Differences in brainstem volume between migraineurs and healthy controls have been demonstrated in previous research, nevertheless, the effect of migraine aura on brainstem volume is still uncertain. The aim of this study was to investigate the brainstem volume in migraineurs and examine the effect of migraine aura on brainstem volume.

Methods: Our study included 90 female migraine patients without white matter lesions. (29 migraine patients with aura (MwA) and 61 migraine patients without aura (MwoA) and 32 age-matched female healthy controls (HC). Using the FreeSurfer image analysis suite, the volumes of the entire brainstem and its subfields (medulla, pons, and midbrain) were measured and compared between migraine subgroups (MwA vs. MwoA) and the healthy control group. The possible effects of migraine characteristics (i.e., disease duration and migraine attack frequency) on brainstem volume were also investigated.

Results: Migraineurs had greater medulla volume (MwoA 3552 ± 459 mm^3, MwA 3424 ± 448 mm^3) than healthy controls (3236 ± 411 mm^3). Statistically, MwA vs. HC $p = 0.040$, MwoA vs. HC $p = 0.002$, MwA vs. MwoA $p = 0.555$. A significant positive correlation was found between disease duration and the volume of medulla in the whole migraine group ($r = 0.334$, $p = 0.001$). Neither the whole brainstem nor its subfields were significantly different in volume between migraine subgroups.

Conclusion: Brainstem volume changes in migraine are mainly localized to the medulla and not specific to the presence of aura.

1. Introduction

Migraine is a common neurological disorder. It affects about 15% of the overall population, and women are two to three times more likely to have migraines [1,2]. Sex differences in migraineurs have been observed not only in prevalence, but also in functional and structural changes [3]. The pathophysiology of migraine is complex and not fully understood. Previous studies have indicated that brainstem plays a critical role in migraine attacks [4,5]. The rostral ventromedial medulla (RVM), the spinal trigeminal nucleus (SpV), and the periaqueductal gray (PAG) are three nociceptive processing sites that are modulated by descending circuitry found in the brainstem [6]. In migraineurs, abnormal

Abbreviations: ANCOVA, analysis of covariance; BOLD, blood oxygenation level dependent; CSD, cortical spreading depression; FLAIR, fluid-attenuated inversion recovery; fMRI, functional Magnetic resonance imaging; HC, healthy controls; ICV, intracranial volume; LCD, least significant difference; 3D, MPRAGE three-dimensional magnetization-prepared rapid gradient-echo; MRI, Magnetic resonance imaging; MwA, migraine patients with aura; MwoA, migraine patients without aura; NRM, nucleus raphe magnus; PAG, periaqueductal gray; RVM, rostral ventromedial medulla; SCP, superior cerebellar peduncle; SpV, spinal trigeminal nucleus; VBM, voxel-based morphometry; WMLs, white matter lesions.

* Correspondence to: Department of Neurology, Medical School, University of Pécs, Rét u. 2, Pécs 7623, Hungary.
E-mail address: pfund.zoltan@pte.hu (Z. Pfund).

https://doi.org/10.1016/j.clineuro.2023.108089
Received 11 November 2023; Received in revised form 13 December 2023; Accepted 15 December 2023
Available online 19 December 2023

brainstem function during attacks suggests that dysfunction in brainstem descending modulatory circuits may play a role in the onset of migraine [7,8]. Neuroimaging studies have demonstrated that migraineurs have functional and anatomical alterations in brainstem regions involved in pain processing [9–11].

It is widely accepted that cortical spreading depression (CSD) accounts for the aura, but mechanisms of triggering CSD in the structurally normal, well-nourished cortex of migraineurs remain unknown. Synaptic drive from subcortical sensory processing structures (brainstem and/or thalamocortical networks) could evoke depolarization of hyperexcitable cortical neurons sufficient to initiate the regenerative spreading depression process [12]. Animal studies demonstrated that the regional blood flow in the brainstem was transiently increased during spreading depression [13]. Additionally, compared to healthy controls, migraineurs have two- to four-fold increased incidence of white matter lesions (WMLs) [14], which are the areas of focal axonal and glial cell injuries in association with decreased intracellular energy metabolism due to impairment of mitochondria [15]. It is plausible that abnormalities in axons passing through these lesions might cause gray matter changes [16].

Recent studies have demonstrated brainstem volume changes in migraine patients [9,11,17,18]. However, findings are partly inconsistent. Structural changes can be sexually dimorphic in migraine and may have an impact on previous studies examining combined male and female groups. Therefore, only females were included in the present study. Moreover, none of the above-cited studies investigated the influence of aura specifically. It was reported that there are white matter microstructural differences between migraine patients with aura (MwA) and migraine patients without aura (MwoA) [19], highlighting the importance of taking the aura into consideration by future migraine studies. In light of hemodynamic change in brainstem during spreading depression [13], we hypothesized that the aura may cause brainstem volume changes in migraineurs as an independent risk factor. Therefore, we investigated migraine patients subdivided into two groups (MwA and MwoA), when assessing the effects of migraine on the volumes of the whole brainstem and its subfields.

2. Patients and methods

2.1. Subjects

A total of 161 female patients fulfilling the International Headache Society (IHS) classification criteria [20] for migraine were initially screened from the Outpatient Headache Clinic of the Department of Neurology, Medical School, University of P é cs, Hungary. All migraineurs included in this study had recurrent headaches, and none of them were on chronic prophylactic therapy before receiving brain MRI scan. Fifty-two of them were excluded due to the presence of white matter lesions (WMLs). In addition, 19 migraine patients without WMLs were excluded because of inadequate MRI quality (i.e., low signal intensity on T1-weighted imaging due to poor slab profile). Eventually, 90 migraine patients were investigated in this study.

The demographic and clinical data of migraineurs were the following: mean age 37.4 ± 11.6, range 18–73 years; disease duration 14.0 ± 11.0, range 1–43 years; attack frequency/month 5.6 ± 4.4, range 0.5–17.0; 61 migraineurs without aura, 29 migraineurs with aura. Migraineurs had no other types of headaches. All migraine patients' headaches or auras were not unilaterally side-locked. An MRI was performed during a headache-free period for each patient. All migraineurs were right-handed based on self-report. As for the healthy controls, 40 age-matched healthy female subjects were included, but only 32 of them (mean age 38.0 ± 10.5, range 19–66 years) eventually were enrolled for the study due to the slab profile issue described above. Healthy controls were recruited by family physicians in Baranya County, Hungary. All healthy controls were right-handed. All control subjects were free of headache, and their brain MRI studies did not show any structural abnormalities. Migraineurs and controls were free of any medical comorbidities.

3. MRI acquisition

All subjects were scanned on the same 3 T MRI scanner (Magnetic TIM Trio, Siemens AG, Erlangen, Germany) using a 12-channel head matrix coil. Beyond the routine T1-weighted, T2-weighted, and FLAIR imaging, a whole-brain T1-weighted 3D MPRAGE sequence was also acquired using the following parameters: TR/TI/TE= 1900/900/3.4 ms; BW= 179 Hz/px; flip angle= 9°; FOV= 210 × 240 mm2, matrix size= 224 × 256, slice thickness= 0.94 mm, 176 axial slices. WMLs were considered if they are visualized as hyperintensities on T2-weighted and FLAIR MRI but without hypointensity on T1-weighted MRI and larger than 3 mm in diameter, appearing on at least two consecutive slices.

4. MR image analysis

Brainstem segmentation was performed on the 3D MPRAGE images using the development version of FreeSurfer image analysis suite released on August 31st, 2017 (https://surfer.nmr.mgh.harvard.edu/fswiki/DownloadAndInstall). The technical details were described previously [21]. Quality control was performed throughout the automatic processing stream. When the reconstruction was inaccurate, error correction was performed based on the recommended workflow (http://surfer.nmr.mgh.harvard.edu/fswiki/Recommended Reconstruction). Freesurfer is based on an algorithm performing automated segmentation and labels the brainstem into its four main subfields (medulla, pons, midbrain, and SCP: superior cerebellar peduncle) shown in Fig. 1**A**. Given that SCP is not strictly part of the brainstem, we did not investigate SCP in this study.

5. Statistical analysis

Statistical analyses were performed using the SPSS 26.0 software (IBM Corp., Armonk, NY, USA). Differences in age between the whole migraine group and healthy controls were assessed by the Mann–Whitney U-test. Age differences among migraine subgroups (MwA and MwoA) and the healthy control group were assessed by the Kruskal–Wallis test followed by pair-wise Mann–Whitney U-test. Differences in continuous migraine-related variables (i.e., disease duration and migraine attack frequency) between migraine subgroups (MwA and MwoA) were compared by Mann–Whitney U-test.

Differences in brainstem and brainstem subfield volumes between whole migraine patients and healthy controls, as well as among three groups (MwA, MwoA and healthy control), were assessed by using ANCOVA with age and total intracranial volume (ICV) as covariates. In cases of statistical significance, least significant difference (LSD) post-hoc analyses were conducted for pairwise comparisons of the three groups. Considering that the MwoA group was significantly older than the MwA group, a closely age-matched (within 2 years) subset of 24 MwoA patients and 24 MwA patients was established and analyzed by ANCOVA.

The possible effects of other migraine characteristics (i.e., disease duration, migraine attack frequency, aura, age, and ICV) on the examined volumes were tested by stepwise multiple linear regression analyses. To control Type I error and achieve great statistical power, the Bonferroni correction was applied for omnibus tests with the number of different brainstem subfields (n = 3). Given that the familywise Type I error probability for LSD procedure remains in the nominal alpha level when comparing three groups [22], it was not necessary for correcting in our post hoc tests [23]. Results were considered significant when *p*-values were less than 0.05.

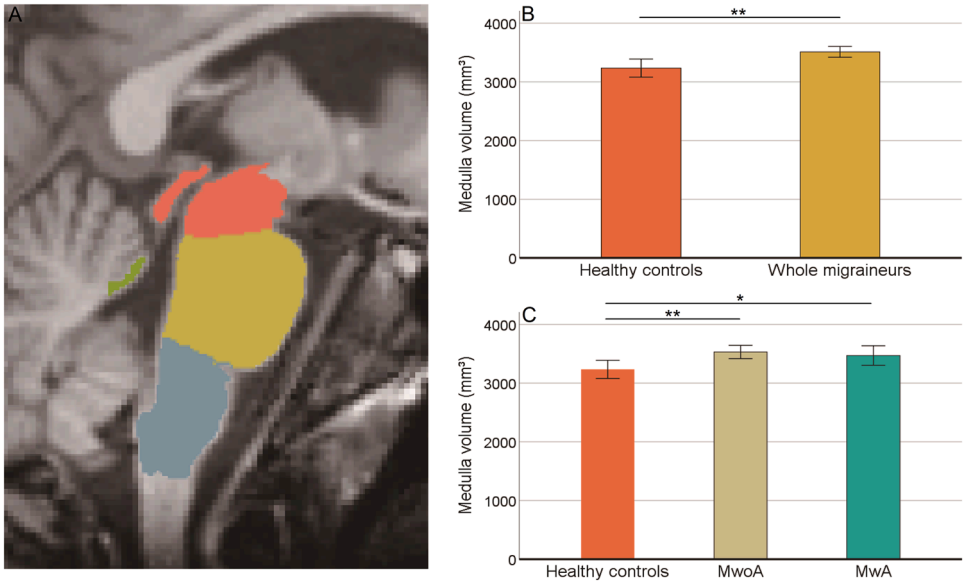

Fig. 1. Segmented brainstem and group differences in medulla volume. MwA: Migraine patients with aura; MwoA: Migraine patients without aura. (A) Subfields of brainstem segmented by FreeSurfer. (B) Medulla volume comparison between healthy controls and all migraineurs. (C) Volume comparisons among migraine subgroups and the healthy control group. Models adjusted for age and total intracranial volume. The error bars indicate standard error. Significant differences between groups are indicated by asterisk, * : $P < 0.05$; ** : $P < 0.01$.

6. Results

There was no significant difference in age between the whole migraine group and healthy controls (2-sided exact *p*-value=0.810). The Kruskal–Wallis test revealed age differences among the two migraine subgroups (MwA and MwoA) and controls (asymptotic *p*-value=0.022). Post hoc testing indicated that the MwoA group was significantly older than the MwA group ($p = 0.006$), but the migraine subgroups were not significantly different in age compared to the control group (Table 1). There was no significant difference in disease duration (2-sided exact p-value=0.113) or migraine attack frequency/month (2-sided exact p-value $p = 0.761$) between the migraine subgroups.

Migraineurs had a greater medulla volume (Fig. 1**B**) compared to healthy controls ($p = 0.002$). The volumes were not different between migraineurs and healthy controls in other structures ($p = 0.761$, 0.826, and 0.599 for the pons, midbrain, and whole brainstem, respectively). There were no significant age*group, age*ICV or group*ICV interactions in the performed analyses.

When comparing our three groups (MwA, MwoA and HC), only the volume of medulla was found to be significantly different among them ($p = 0.009$, Table 2). Post-hoc testing indicated that both the MwA and the MwoA groups had bigger medulla (Fig. 1**C**) compared to the HC group ($p = 0.040$ and $p = 0.002$, respectively), while the two migraine subgroups were not different in medulla volume ($p = 0.555$). Closely age-matched MwoA and MwA showed no significant difference either ($p = 0.943$, 0.251, 0.971 and 0.836 for whole brainstem, medulla, pons and midbrain volumes, respectively). Focusing on the whole migraine group, the stepwise linear regression showed a significant positive correlation (Fig. 2) between disease duration and the medulla volume ($r = 0.334$, $p = 0.001$; no other potential independent variables were included in the model by the stepwise method). For other brainstem structures, no migraine characteristics were chosen by the stepwise

Table 2
Group differences in brainstem and subfield volume.

	Groups			ANCOVA test	
	Aura+	Aura-	Controls	F (df1, df2)	*P* (uncorrected)
Brainstem (mm^3)	22276 ± 2008	22407 ± 1793	22119 ± 2180	0.376 (2117)	0.688
Medulla (mm^3)	3424 ± 448	3552 ± 459	3236 ± 411	4.926 (2117)	0.009[b]
Pons (mm^3)	13146 ± 1365	13216 ± 1325	13234 ± 1591	0.383 (2117)	0.683
Midbrain (mm^3)	5455 ± 399[a]	5424 ± 382	5424 ± 509	0.135 (2116)[a]	0.874[a]

Aura+ : Migraine patients with aura; Aura-: Migraine patients without aura; Controls: Healthy controls.
Volumes are presented as uncorrected mean ± standard deviation.
[a]One subject was excluded due to the corresponding standardized residuals from the ANCOVA model being below − 3 or above 3.
[b]The uncorrected *P*-value survived Bonferroni correction.

Table 1
Demographic and clinical data of migraine patients and healthy controls.

	Aura+ ($n = 29$)	Aura- ($n = 61$)	Controls ($n = 32$)	Differences (*P* value)		
				A+ vs. A-	A+ vs. C	A - vs. C
Age (years)	32.4 ± 10.8 (18-50)	39.8 ± 11.3 (18-73)	38.0 ± 10.5 (19-66)	0.006[a]	0.066[a]	0.493[a]
Disease duration (years)	11.4 ± 10.1 (1-33)	15.3 ± 11.3 (1-43)	-	0.113[b]	-	-
Migraine attack frequency/month	5.7 ± 4.6 (5-15)	5.5 ± 4.4 (1-17)	-	0.761[b]	-	-

Aura+ /A+ : Migraine patients with aura; Aura-/A-: Migraine patients without aura; Controls/C: Healthy controls.
Values are given as mean ± standard deviation (minimum-maximum).
[a]The statistical *P* values were based on post-hoc Dunn's test.
[b]The statistical *P* values were based on Mann–Whitney U-test (2-sided exact *p*-value).

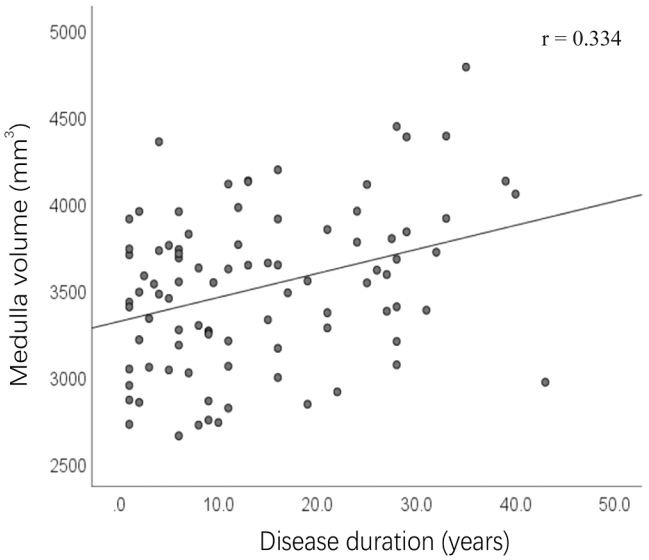

Fig. 2. Scatter plot showing that disease duration and medulla volume have a significant positive correlation.

method as significant predictors of the volume.

7. Discussion

In the present study, we investigated a rather homogeneous migraine group to explore the brainstem volume alteration in female migraineurs as well as the potential effects of aura and other migraine characteristics on brainstem volume. Our results demonstrated that migraineurs had greater medulla volume compared to healthy controls, and also suggested that aura is not the primary cause of brainstem volume changes.

Previous imaging studies comparing the two subgroups of migraine (i.e., MwA and MwoA) were mostly conducted using perfusion or functional magnetic resonance imaging (fMRI) techniques [24–28]. An fMRI study found no significant differences in the executive control network (commonly including prefrontal cortex, frontopolar cortex, anterior cingulate cortex and posterior parietal cortex) functional connectivity between the MwA and MwoA groups [24]. Interestingly, Datta and colleagues found higher blood oxygen level–dependent (BOLD) fMRI response to visual stimulus within the occipital pole in MwA compared to MwoA, but resting cerebral blood flow of primary visual cortex was not different between these two groups [25]. Additionally, during an interictal phase, Kreczmański et al. found BOLD fMRI activity differences in response to visual stimuli within the brainstem of MwA compared to MwoA [26]. However, another perfusion-weighted imaging study indicated that during the aura there were no hemodynamic changes in the brainstem or other anatomical areas except the occipital cortex [27]. Based on the abovementioned studies, we assume there are subtle functional differences in the brain between MwA and MwoA groups (especially in the occipital lobe and brainstem), which may also be associated with morphological changes. However, functional data obtained from brainstem are affected by technical challenges that can influence the quality of the results, which also limits its use in this region [28]. In the present study, we did not find a significant difference in the volume of the whole brainstem and its subfields between MwA and MwoA. Future studies combining morphological and functional measures are needed to further investigate the brainstem and assess the potential correlation between these modalities. Especially, longitudinal studies could provide more information as to whether functional changes precede morphological alterations and whether they indicate findings in the same part of the brainstem.

Central sensitization may contribute to migraine due to atypical modulation of pain by the descending pain modulatory system [29–31]. The medulla is the brain region with high density of areas involved in pain modulation, including the RVM, the nucleus of the solitary tract, dorsal reticular nucleus, ventral reticular nucleus and ventrolateral medulla [32]. Excitatory midbrain nuclei and inhibitory medulla nuclei compose a circuit that controls cortical arousal levels (e.g., cognition and pain) [33]. A previous study [17] found that migraineurs had smaller midbrain volumes. Combined with our finding that migraine patients had greater medulla volumes, these structural changes might be the result of a disrupted regulation of the circulatory system. In addition, PAG dysfunction has also been implicated in different pain diseases including migraine [34]. fMRI studies found that PAG had significant functional connectivity with RVM [35]. The PAG influences descending pain modulation primarily through its reciprocal connections with the RVM [36]. Our results are consistent with previous studies indicating evidence for a medullary brainstem mediator of pain [32–36]. Pain modulation from the medulla is the only part of the endogenous pain control system from which facilitation of pain transmission can be produced along with antinociception [37]. The brainstem nuclei in the RVM exclusively facilitate nociceptive spinal activity [38]. Edelmayer et al. conducted an animal experiment which demonstrated that injection of bupivacaine directly into the RVM could attenuate allodynia induced inflammatory mediators [39]. Another recent animal experiment showed that injection of neuronal excitant into nucleus raphe magnus (NRM) and PAG selectively inhibited the responses of trigeminovascular second-order neurons to dura mater; meanwhile, injection of local anesthetic into these two nuclei selectively potentiated the responses of these neurons to dura [40]. RVM is a key structure in descending pain modulation, whereas the main function of the NRM is mostly pain mediator. These findings suggest that volume alteration may contribute to dysfunction of pain modulation in medulla. Besides, we found that disease duration and medulla volume had a significant positive correlation, this supports a progressive increase of medulla volumes with long disease duration, but longitudinal studies are needed to prove in the future.

To date, there have been very limited morphological studies on the brainstem of migraineurs, and the findings from these studies are variable [9,11,17,18]. We did not find significant volume difference for the whole brainstem in migraine patients compared to healthy controls, which is in line with a previous study [17]. Nevertheless, Petrusic et al. found that MwA had a greater whole brainstem volume compared to healthy controls, and increased volumes were seen in pons and midbrain as well [18]. However, this study only included MwA, which may partly contribute to the different results. Furthermore, previous studies were mostly conducted in migraine groups including both males and females [17,18], while our study included only females. Maleki and colleagues have found stronger responses to noxious stimulation in SpV of the brainstem for female migraineurs relative to male migraineurs [3], which may be associated with sex differences in brainstem volume. The trigeminal nerve is thought to be involved in the pathophysiology of migraine, and SpV receives afferents from trigeminal nerve, which may lead to the volume changes of SpV in migraineurs. Two subsequent studies found reduced grey matter volume in the SpV in migraine patients [9,11], but, unfortunately, neither of them investigated the effect of gender on their findings. SpV is a small nucleus/tract located in the lateral medulla of the brainstem. Despite the greater volume of medulla found in present study, we could not determine if our results are conflicting, because we did not examine any subregions of the medulla. Future studies using a higher spatial resolution and specific image contrast – that makes possible the differentiation of medulla subregions – need to be performed to reliably assess morphological differences separately in each subregion. Additionally, these two studies used voxel-based morphometry (VBM) approach, which could lead to different findings from those obtained by FreeSurfer [41].

It is worth noting that none of the abovementioned studies investigated the impact of aura on brainstem volume. Although most of them excluded subjects with abnormal findings on T1 and T2 MR images,

migraine aura could cause white matter microstructural differences [19], which are invisible on routine clinical MR images but may still contribute to volume alteration of brain structures.

Beyond sex, aura and various other factors such as different image processing methods (VBM vs. FreeSurfer), and the different number of study participants could also contribute to inconsistent findings from previous studies. In this context, it should be emphasized that our sample size is bigger and more homogeneous compared to most previous studies.

Although our study suggests that aura has no obvious effect on brainstem volume changes, we did not obtain an fMRI scan on the same group to investigate the functional abnormalities. Moreover, the number of MwA is not sufficient for us to explore the impact of different types of migraine aura on brainstem volume, which needs to be considered for future studies. Additionally, because the number of MwoA with WMLs is relatively small, we did not investigate the effect of lesions on brainstem volume changes, which needs to be taken into consideration by future studies to better understand the conflicting findings from previous studies.

8. Conclusion

We investigated a group of female migraineurs and found that they have greater medulla volumes compared to healthy controls, irrespective of aura. There is no significant volume difference in the whole brainstem or its subfields between MwA and MwoA.

Funding

This work was supported by grants from EEA/Norwegian Financial Mechanism HU 0114 - “Save what can be saved” - applied neurological research using high-field magnetic resonance imaging, by TÁMOP-4.2.1/B, EFOP-3.6.2–16-2017–00008, “The role of neuroinflammation in neurodegeneration: From molecules to clinics”, TKP2021-EGA-16 and TKP-2021-EGA-13. Project No. TKP2021-EGA-16 and TKP-2021-EGA-13 have been implemented with the support provided from the National Research, Development and Innovation Fund of Hungary, financed under the TKP2021-EGA funding scheme. This work was also supported by the Hungarian Brain Research Program 2 and 3 (2017–1.2.1-NKP-2017–00002 and NAP 3.0) and the Translational Neuroscience National Laboratory (RRF-2.3.1–21-2022–00011). Z. P. was funded by PTE ÁOK-KA-2017–23. EFOP-3.6.3-VEKOP_00009.

CRediT authorship contribution statement

Conception and design: Zoltán Pfund, Mingchen He, Gábor Perlaki. Acquisition of data: Zoltán Pfund, Gábor Perlaki, Edit Bosnyák, Renáta Rozgonyi, Flóra John, Anita Trauninger, Gréta Kis-Jakab. Analysis and interpretation of data: Gábor Perlaki, Gergely Orsi, Mingchen He, Hedvig Komáromy. Drafting the manuscript: Gábor Perlaki, Mingchen He, Zoltán Pfund. Revising it for intellectual content: Zoltán Pfund, Gábor Perlaki, Anita Trauninger, Flóra John, Kata Eklics. Final approval of the completed manuscript: Mingchen He, Gréta Kis-Jakab, Hedvig Komáromy, Gábor Perlaki, Gergely Orsi, Edit Bosnyák, Renáta Rozgonyi, Flóra John, Anita Trauninger, Kata Eklics, Zoltán Pfund.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Acknowledgements

Not applicable.

Ethics approval

All subjects provided informed consent to study participation. Studies were performed in accordance with the approval (4022) of the Regional Research Ethics Committee of the Medical Center, Pécs.

Consent for publication

The publication was accepted by all authors.

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