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Longitudinal changes in neurometabolites in pediatric migraine across child development: a pilot study

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Migraine severely impacts quality of life in children and adolescents and has been identified as the most important cause of neurological disability in youth. There is evidence that an imbalance between brain excitation (glutamate) and inhibition (GABA) is one of the driving factors behind migraine attacks. During adolescence, changes to glutamatergic and GABAergic neurons occur. It is therefore perhaps unsurprising that adolescence is also a transitory time for migraine, with changes in incidence and associated symptoms. However, there is a lack of research into interactions between brain chemistry changes and progression of migraine during the adolescent period. In this longitudinal study, magnetic resonance spectroscopy (MRS) was used to measure metabolite levels in the thalamus, sensorimotor cortex and visual cortex over two sessions (on average 3 years apart) in participants ages 7-18y. We show that a decrease in thalamic GABA levels over time is related to new onset migraine in previously unaffected adolescents (t(41.4) = 2.79, *p* = 0.008) and increased disease burden in those who previously experienced migraine (b=-0.01, SE = 0.003, *p* < 0.001). This provides new evidence for a role of thalamic GABA early in migraine onset and progression, and a potential target for new treatments.

Keywords Migraine, Pediatric, GABA, Glutamate, Magnetic resonance spectroscopy (MRS), Macromolecule-suppressed GABA

Migraine is a neurological disorder characterized by attacks of recurrent, severe headaches. Migraine often begins in childhood and is the most frequent primary headache disorder in youth brought to medical attention, affecting nearly 10% of youth worldwide[1]. Migraine severely impacts quality of life, with youth with migraine reporting an impact on quality of life similar to that reported by children with cancer and rheumatologic diseases. The pain and associated symptoms disrupt home, school and social lives, and youth with migraine are more likely to experience depression, anxiety and behavioural challenges[2]. This often continues into adulthood, with over half of youth with migraine still experiencing attacks 5–7 years later[2]. Despite its high prevalence and severe impact, there are still limited treatment options for migraine in youth, in part due to a lack of understanding of the pathophysiology.

There is evidence that an imbalance between excitation and inhibition in the brain and an inability to maintain homeostasis could be one of the driving factors of attacks of migraine. This altered balance may specifically disrupt signaling between the thalamus and the cortex[3]. The primary role of the thalamus is to transfer sensory information between the peripheral nervous system and multiple cortical areas, including the sensory and visual cortices[4]. Indeed, in addition to being sensitive to light and sound during the attack, individuals with migraine have altered sensory processing and atypical multisensory integration between attack periods[5]. Magnetic resonance imaging (MRI) studies have shown increased activation in sensory and visual cortices in response

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to stimuli in individuals with migraine[6] as well as altered functional connectivity in these regions at rest[7], and altered thalamic connectivity[4]. EEG studies have shown altered alpha and gamma band oscillations in sensory and visual cortices, potentially due to abnormal thalamocortical control[3].

Magnetic resonance spectroscopy (MRS) studies have shown evidence of alterations in excitatory (glutamate) and inhibitory (GABA) neurotransmitters in key brain regions of individuals with migraine. Cross sectional studies have shown altered levels of glutamate in the thalamus[8] and occipital cortex[9–11]. Levels of GABA in the sensory cortex have been associated with migraine characteristics in youth, such as symptoms and years with the disease[11]. More recently, Cho et al. (2024) collected MRS data in adolescents with multiple samples in a short time period (four samples within a two-week period) to characterize neurometabolite changes leading up to and following a migraine attack. In the time leading up to a migraine (24–72 h preceding), they found decreases in GABA levels in the thalamus and glutamate levels in the occipital and sensory cortices. In contrast, GABA levels in the sensory cortex increased. Following the migraine (24–72 h following), glutamate levels in the occipital and sensory cortex continued to decrease, as well as glutamate levels in the thalamus[12]. This highlights the important role these neurotransmitters play in migraine pathology, however there is a lack of knowledge regarding the role of neurotransmitter changes with the evolution of this neurological disorder over longer time spans (and development).

Adolescence is a transitory time for migraine; the prevalence of migraine increases with age, with a high incidence in late adolescence and peaking in early adulthood. During adolescence the sex ratio of incident migraine cases also appears to change (i.e., incident migraine becomes much more prevalent in females)[14], but the mechanism behind these changes is not understood. During typical adolescent development, there are important changes in the neurotransmitter profile, including changes to glutamatergic and GABAergic neurons, resulting in an "excitability shift"[13]. Therefore it is perhaps unsurprising that migraine, which is likely driven by excitability changes, also changes during this time. However, the relationship between changes in glutamate and GABA levels and progression of migraine during this period of brain development has yet to be investigated.

In this preliminary longitudinal study, we used MRS to measure GABA and glutamate levels in the thalamus, sensorimotor cortex, and occipital cortex in youth with and without migraine at two time points, on average three years apart. While many regions are implicated in migraine pathophysiology, we chose to focus on the sensorimotor cortex as a primary region involved in pain and sensory processing, the occipital cortex for its role in visual processing and aura, and the thalamus for its role in thalamocortical control and sensory integration. Several other metabolites can also be quantified from MRS (N-acetylaspartate (NAA), Creatine (Cr), Choline (Cho), and myo-inositol (Ins)), which were investigated in a secondary analysis. This study provides new insight into the interactions between neurochemical changes across development and progression of migraine, directing new research and potential therapeutic options.

Methods

Ethics statement

This study was approved by the Conjoint Health Research Ethics Board (CHREB), University of Calgary (REB23-0538). All procedures were performed in accordance with CHREB guidelines and regulations and conformed to the Declaration of Helsinki, and all the study participants provided informed consent/assent and their parents/guardians provided informed consent at time of enrollment.

Participants

This study recruited participants from previous imaging studies on pediatric migraine[11,12], including both participants with migraine and control participants. This approach facilitates the longitudinal study of pediatric migraine as development appears to impact the presentation of migraine. Participants were invited to participate in a second MRI session at least 6 months following their first visit, and only those who consented to participate are included in the subsequent analysis. All data were collected at the Alberta Children's Hospital, with the second visit occurring between July 2023 and November 2023. Exclusion criteria for participation included contraindications to MRI, inability to read or understand English, concussion within the past three months, neurological, psychiatric, or systemic disorders (e.g., attention deficit hyperactivity disorder (ADHD), autism, developmental delay, intellectual disability psychosis, schizophrenia) or major comorbidities such as epilepsy or arthritis.

Control Group – Fifteen children and adolescents aged 7–17 returned for a second scan (recruited from Bell et al. (2021)[11]. Controls were originally recruited using the Healthy Infants and Children Clinical Research Program (HICCUP) at the Alberta Children's Hospital. See Bell et al. (2021)[11] for further details.

Migraine Group – Twenty-two children and adolescents aged 7–17 with a diagnosis of migraine returned for a second scan (recruited from Bell et al. (2021)[11] and Cho et al. (2024)[12]. Participants were originally recruited from the Vi Riddell Pain Clinic and headache clinics at the Alberta Children's Hospital and the local community. Participants were included if they had received a physician diagnosis of migraine (with or without aura), which was confirmed using the International Classification of Headache Disorders 3rd Edition (ICHD-3) beta diagnostic criteria[15]. See Bell et al. (2021)[11] and Cho et al. (2024)[12] for specific inclusion criteria.

At the second timepoint, all participants (control and migraine) completed standardized headache questionnaires inclusive of questions previously validated against ICHD criteria[16], and a headache neurologist reviewed the data, classifying participants as either meeting criteria for migraine with or without aura based on the ICHD-3 criteria, or not meeting any criteria for migraine. Participants were then classified into three groups: (1) control participants who remained controls (Con-Con), (2) participants with migraine who continued to have migraine (Mig-Mig), (3) initially control participants who now experience migraine (Con-Mig). There were no participants with migraine at the first visit who no longer experienced migraine at the second visit. A flow chart of participant numbers can be seen in Fig. 1.

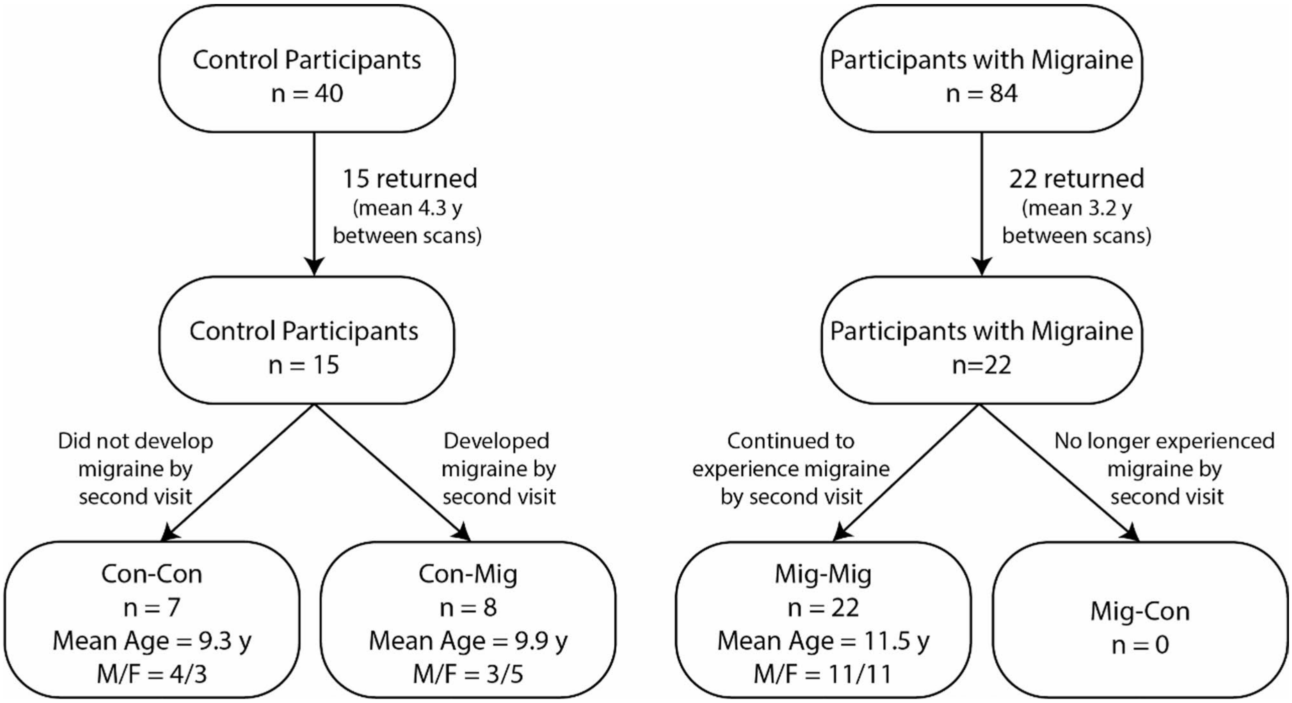


Fig. 1. Flow chart showing the study cohort. Control participants were all recruited from Bell et al. (2021)[11]. Participants with migraine were recruited from Bell et al. (2021)[11] and Cho et al. (2024)[12].

Questionnaires

At both timepoints, participants completed the Pediatric Migraine Disability Assessment (PedMIDAS) questionnaire[17]. The PedMIDAS is a pediatric version of the self-report Migraine Disability Assessment (MIDAS) questionnaire commonly used in adults, and has been validated in children and adolescents ranging from 6 to 18[17]. Larger scores indicate more headache related disability, with scores ranging from 0 to 240. Participants were also asked how many days per month do they have headache, to quantify headache frequency.

MR acquisition

All data were acquired on a 3 T GE 750w MR scanner using a 32-channel head coil. A T1-weighted anatomical image was collected for voxel placement (BRAVO; 230 slices, TE/TR = 2.7/7.4 ms, 1mm^3 isotropic voxels, matrix 240 × 240). GABA-edited spectroscopy data were collected using macromolecule-suppressed MEGA-PRESS (TR/TE = 1800/80 ms, 20 ms editing pulses at 1.9 and 1.5 ppm, 256 averages). Separate PRESS data (TR/TE = 1800/35 ms, 64 averages) were acquired to quantify glutamate[18]. Voxel sizes are described below.

MRS data collection for the thalamus and sensorimotor cortex were identical for all cohorts and timepoints. 3 × 3 × 3 cm^3 voxels were placed in the thalamus (midline centered) and right sensorimotor cortex (the hand-knob of the motor cortex was used for initial localization and then the voxel was centered between the precentral gyrus and postcentral gyrus; the voxel was then rotated such that the coronal and sagittal planes aligned with the cortical surface[19], Fig. 2). For participants recruited from Bell et al. (2021)[11], a 3 × 3 × 3 cm^3 voxel was also placed in the occipital cortex, midline centered, as close to aligning with the parieto-occipital sulcus as possible, without including cerebellum (Fig. 2). MEGA-PRESS and PRESS data were acquired using the parameters described above. For participants recruited from Cho et al. (2024)[12], a 2 × 2 × 2cm^3 voxel was placed in the occipital cortex, and only PRESS data was acquired (TR/TE = 1800/30 ms, 112 averages). This was a result of different protocols in the original studies. Data collection at timepoint 2 was matched to collection at timepoint 1, i.e., those who had occipital GABA collected at timepoint 1 followed the same procedure at timepoint 2, those who only had PRESS data from a smaller voxel at timepoint 1 followed the same procedure at timepoint 2. While we recognize the occipital voxel placement differs between the two voxel sizes, to increase the sample size for the glutamate data in the occipital cortex we chose to aggregate these data.

MRS processing and quantification

MEGA-PRESS data were analyzed using Gannet 3.3.2[20], which included the following preprocessing steps: coil combination, frequency and phase correction, apodization, and down weighting of motion-corrupted averages. Voxel tissue fractions were calculated by generating subject-specific voxel masks which were then registered to each individual tissue segmented T1 image using spm12[21]. These fractions were then used for correction of tissue-specific water visibility, as well as T1 and T2 relaxations of both water and metabolites. Tissue corrected GABA is reported using the alpha-correction, which assumes twice as much GABA in grey matter than white

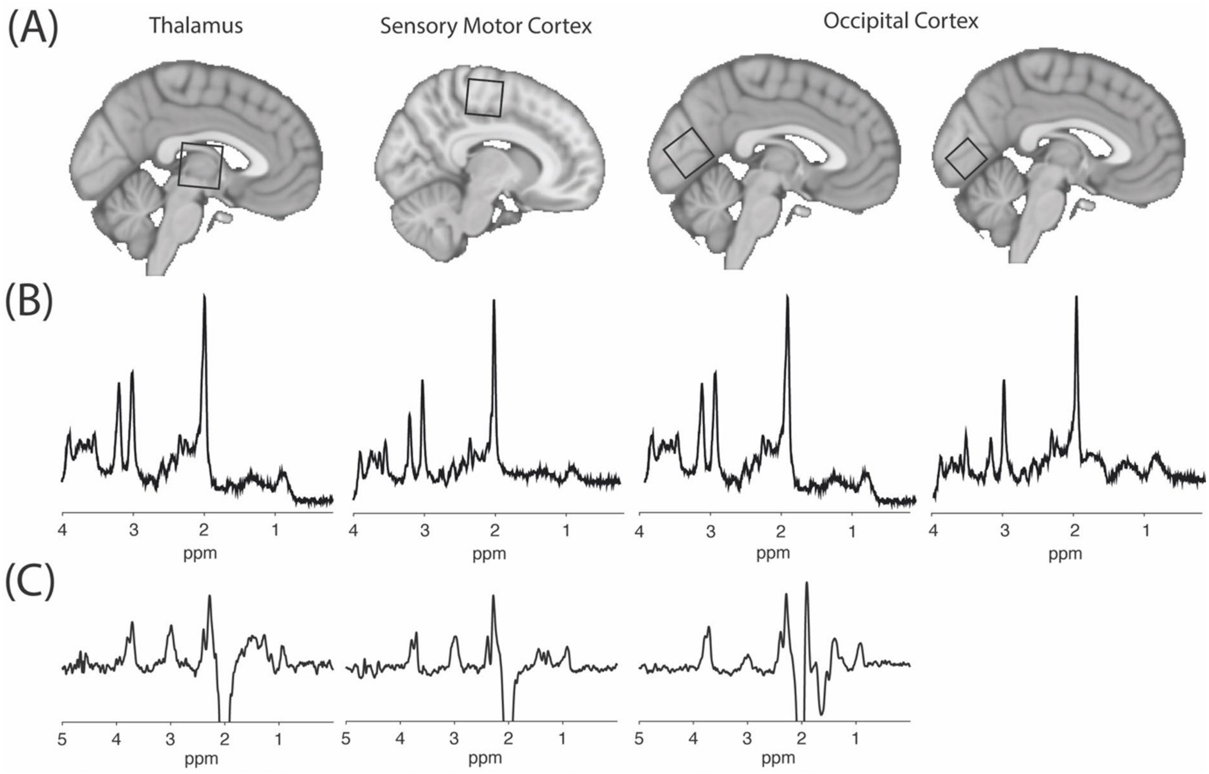


Fig. 2. (**A**) Example voxel placement. 3 × 3 × 3 cm^3 voxels were placed in the thalamus (midline centered) and right sensorimotor cortex. Depending on the data acquired at timepoint 1, either a 3 × 3 × 3 cm^3 voxel or a 2 × 2 × 2cm^3 voxel was placed in the occipital cortex. MEGA-PRESS and PRESS data were acquired from 3 × 3 × 3 cm^3 voxels. PRESS data only were acquired from 2 × 2 × 2cm^3 voxels. (**B**) Example PRESS data from each location. (**C**) Example macromolecule suppressed MEGA-PRESS data from each location.

matter[22]. Data quality was assessed by visual inspection and fit error, with spectra with a fit error over 15% excluded.

PRESS data were preprocessed with the FID-A toolbox[23] using the following preprocessing steps: coil combination, removal of motion-corrupted averages, frequency drift correction, and zero-order phase correction[24]. LCModel Version 6.3–1 N was used to apply eddy current correction and quantification relative to water. Basis sets for quantification (including alanine, aspartate, choline, glycerophosphocholine, phosphocholine, creatine, phosphocreatine, GABA, glutamate, glutamine, lactate, inositol, N-acetyl aspartate, N-acetylaspartylglutamate, scyllo-inositol, glutathione, glucose, taurine, bHb, Citrate, Ethanol, Glycine, PE) were simulated using the FID-A toolbox based on exact sequence timings and RF pulse shapes. Metabolite values were corrected for tissue composition, including tissue-specific water density, and T1 and T2 relaxations of both water and metabolites[24,25] using the tissue fractions previously generated. Glutamate was quantified as a combination (Glx) of glutamate and its precursor, glutamine. Glutamate and glutamine overlap on the spectra due to their similar chemical compositions; subsequently, it is difficult to separate the individual signals. Data quality was assessed by visual inspection and metabolite linewidth, and spectra with a linewidth over 0.1 ppm were excluded.

Statistical analysis

Statistical analyses were conducted using R (version 4.1.2, R Core Team (2020) https://www.R-project.org/[26]. Participant characteristics (age and PedMIDAS) and quality metrics (linewidth, SNR) were statistically compared between groups with ANOVAs using the "aov" function in the "stats" package (version 4.1.2).

To test for changes in neurochemical levels over time, linear mixed-effects models were run using the "lmer" function in the "lme4" package (version 1.1.27.1)[27], with timepoint (1 or 2) and group (Con-Con, Mig-Mig, Con-Mig) included as fixed effects (including a timepoint by group interaction), and subject included as a random effect. Age and sex were also included in the model as fixed effects (notation: lmer(metabolite ~ timepoint * group + (1|subject) + age + sex)). The time between scans was not included in this model due to the inclusion of age at each timepoint. Significant models were further explored using pairwise comparisons implemented using the "emmeans" function (version 1.10.3)[28].

A similar linear mixed effect model was also used to test for differences in neurochemical levels over time by aura status (notation: lmer(metabolite ~ timepoint * status + (1|subject) + age + sex)). As above, significant models were further explored using pairwise comparisons implemented using the "emmeans" function[28].

Preventative Medication	
Amitriptyline	1
Botox	1
Magnesium	2
Coenzyme Q10	3
Riboflavin	1
Abortive Medication	
Ibuprofen	16
Acetaminophen	1
Naproxen	4
Rizatriptan	3
Sumatriptan	1
Cambia	2
Ondansetron	2

Table 1. List of preventative and abortive medications taken by participants with migraine.

		Con-Con	**Mig-Mig**	**Con-Mig**	***P* value**
	***N* (Female)**	**7 (3)**	**22 (11)**	**8 (5)**	
	Aura	n/a	12	0	
	Family history of migraine	2 (29%)	19 (86%)	6 (75%)	
	Time between Scans (months)	52.14 (4.56)	38.86 (17.14)	51.25 (5.01)	**0.03**
Timepoint 1	Age (years)	9.29 (1.89)	11.45 (2.79)	9.88 (1.89)	0.66
	No. attacks per month	n/a	7 (6)	n/a	
	PedMIDAS	1.71 (1.50)	27.32 (43.08)	2.38 (5.55)	**0.10**
	PRESS LW (Hz)	7.83 (1.97)	8.98 (2.63)	8.31 (1.53)	0.55
	MEGA-PRESS LW (Hz)	10.89 (1.42)	11.58 (1.98)	11.71 (1.43)	0.15
Timepoint 2	Age (years)	13.57 (2.07)	14.41 (2.20)	14.13 (1.81)	0.09
	No. attacks per month	0	8 (6)	5 (5)	
	PedMIDAS	0.57 (1.51)	21.01 (29.43)	7.50 (7.91)	**0.10**
	PRESS LW (Hz)	7.87 (1.95)	9.50 (3.19)	9.13 (3.62)	0.19
	MEGA-PRESS LW (Hz)	11.14 (1.70)	11.56 (2.06)	11.25 (1.86)	0.84

Table 2. Participant characteristics and quality metrics by migraine status, mean (SD). PedMIDAS: Pediatric Migraine Disability Assessment; LW: Linewidth.

Linear models were used to test for relationships between the change in metabolite level and the change in PedMIDAS score using the "lm" function (notation: lm(change in metabolite ~ group + change in pedMIDAS + months between scans + sex + age at timepoint 1), as well as for relationships between metabolite levels in timepoint 1 and the change in PedMIDAS score (notation: lm(metabolite ~ group + change in pedmidas + months between scans + sex + age at timepoint 1). Similar analyses were performed for the change in migraine frequency (number of migraine days in a month).

This was a convenience sample and therefore no a priori sample size calculations were conducted. The Bonferroni adjustment was used to correct for multiple comparisons of the three brain regions. Both the uncorrected (p) and the Bonferroni corrected (p_B) values are reported.

As different voxel sizes were collected in the OCC, we performed confirmatory analyses using only data from the larger voxel size (as this made up the majority of cases). As this did not change our findings, these results are not reported.

Results

Preventative and abortive medications used by the participants with migraine are reported in Table 1. Demographics are reported in Table 2 for the three groups: (1) control participants who remained controls (Con-Con), (2) participants with migraine who continued to have migraine (Mig-Mig), (3) initially control participants who now experience migraine (Con-Mig). There were no participants with migraine at the first visit who no longer experienced migraine at the second visit.

Neurochemical changes over time

Thalamus

GABA levels in the thalamus showed a significant effect of group (F(1,26.6) = 4.95, $p = 0.01$, $p_B = 0.03$) and a significant group by timepoint interaction (F(2,26.6) = 6.10, $p = 0.007$, $p_B = 0.021$). Pairwise comparisons showed a significant decrease between timepoint 1 and 2 in the Con-Mig group only (t(41.4) = 2.79, $p = 0.008$; Fig. 3A).

tCho levels in the thalamus showed a significant effect of group (F(1,32.2) = 5.94, $p = 0.006$, $p_B = 0.018$), but no effect of time or a time by group interaction. Pairwise comparisons showed that, relative to the Con-Con group, tCho levels were significantly lower in the Mig-Mig group (t(32.1) = 3.42, $p = 0.005$) and the Con-Mig group (t(31.7) = 2.45, $p = 0.05$; Fig. 3B).

tCr levels in the thalamus also showed a significant effect of group, although this did not survive correction for multiple comparisons (F(2,32.7) = 3.78, $p = 0.03$, $p_B = 0.09$). Pairwise comparisons showed that tCr levels were significantly lower in the Mig-Mig group compared to the Con-Con group (t(33.0) = 2.70, $p = 0.03$; Fig. 3C).

There were no significant changes in Glx, tNAA or Ins in the thalamus.

Sensorimotor cortex

There was a significant effect of time on tNAA levels in the sensorimotor cortex, although this did not survive correction for multiple comparisons (F(1,63.8) = 5.30, $p = 0.02$, $p_B = 0.06$).

There were no other significant changes in neurochemical levels in the sensorimotor cortex.

Occipital cortex

There was a significant effect of group on tCho levels in the occipital cortex, although this did not withstand correction for multiple comparisons (F(2,24.8) = 3.30, $p = 0.05$, $p_B = 0.15$). Pairwise comparisons showed a non-significant difference between Con-Con and Con-Mig only (t(23.8) = 2.44, $p = 0.06$; Fig. 4).

There were no other significant changes in neurochemical levels in the occipital cortex.

Effects of aura status on neurochemical changes

Of the 31 participants who experienced migraine (from both the Mig-Mig and Con-Mig groups), 22 experienced migraine without aura and 9 experienced migraine with aura.

Thalamus

Aura status had a significant effect on levels of tCho and tCr in the thalamus (tCho: F(2,34.6) = 7.39, $p = 0.002$, $p_B = 0.006$; tCr: F(2,34.5) = 5.37, $p = 0.009$, $p_B = 0.027$). Pairwise comparisons showed tCho levels in controls were significantly higher than migraine with aura (t(34.4) = 3.83, $p = 0.002$) and migraine without aura (t(32.9) = 2.42, $p = 0.05$). tCr levels were significantly higher in controls compared to migraine with aura (t(34.8) = 3.27, $p = 0.007$).

There were no other significant differences in neurochemical levels in the thalamus between participants with and without aura.

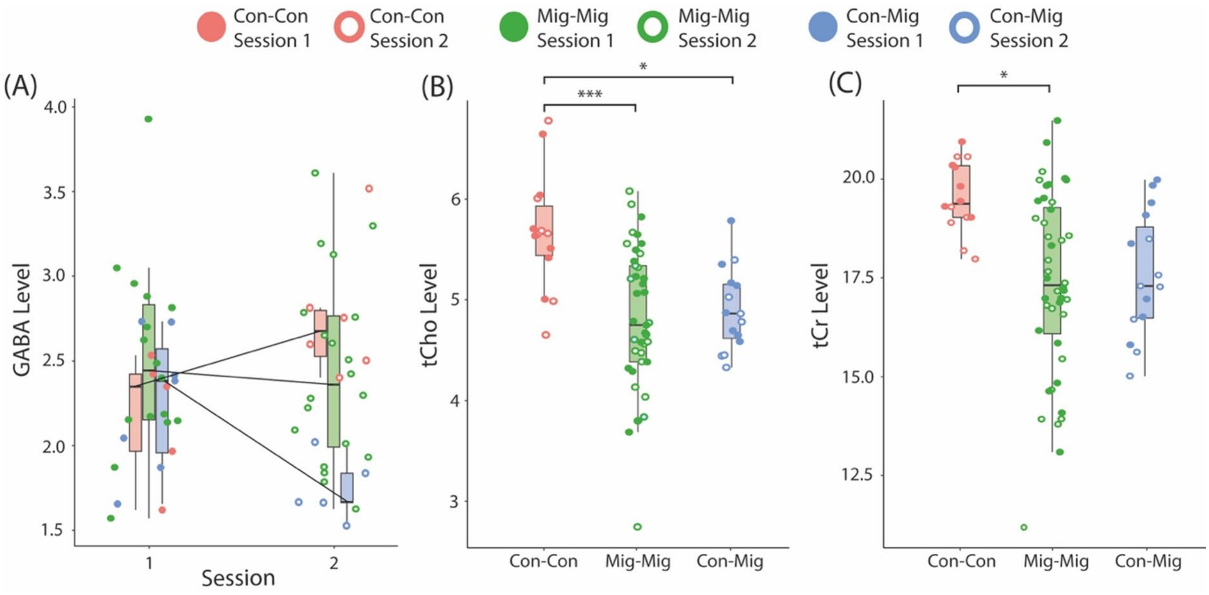


Fig. 3. Thalamic metabolite levels across time and groups. (**A**) Significant group by timepoint interaction on GABA levels in the thalamus (F(2,26.6) = 6.10, $p = 0.007$, $p_B = 0.021$). Pairwise comparisons showed a significant difference between timepoint 1 and 2 in group Con-Mig only (t(41.4) = 2.79, $p = 0.008$). (**B**) Significantly lower thalamic tCho levels in the Mig-Mig (t(32.1) = 3.42, $p = 0.005$) and Con-Mig (t(31.7) = 2.45, $p = 0.05$) groups compared to the Con-Con group. (**C**) Significantly lower thalamic tCr levels in the Mig-Mig group compared to the Con-Con group (t(33.0) = 2.70, $p = 0.03$, $p_B = 0.09$).

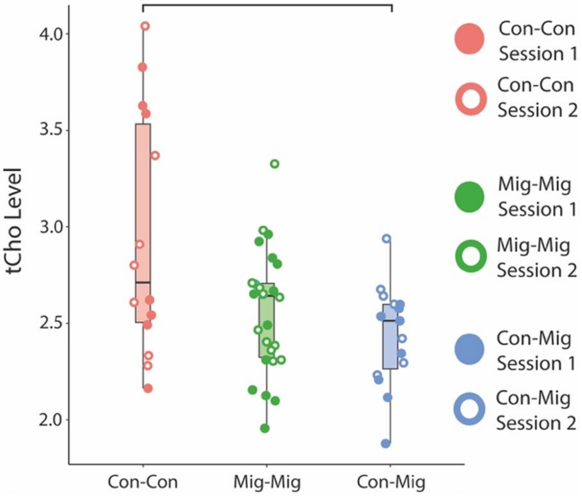


Fig. 4. Lower tCho levels in the occipital cortex in the Con-Mig group compared to the Con-Con group (t(23.8) = 2.44, p = 0.06).

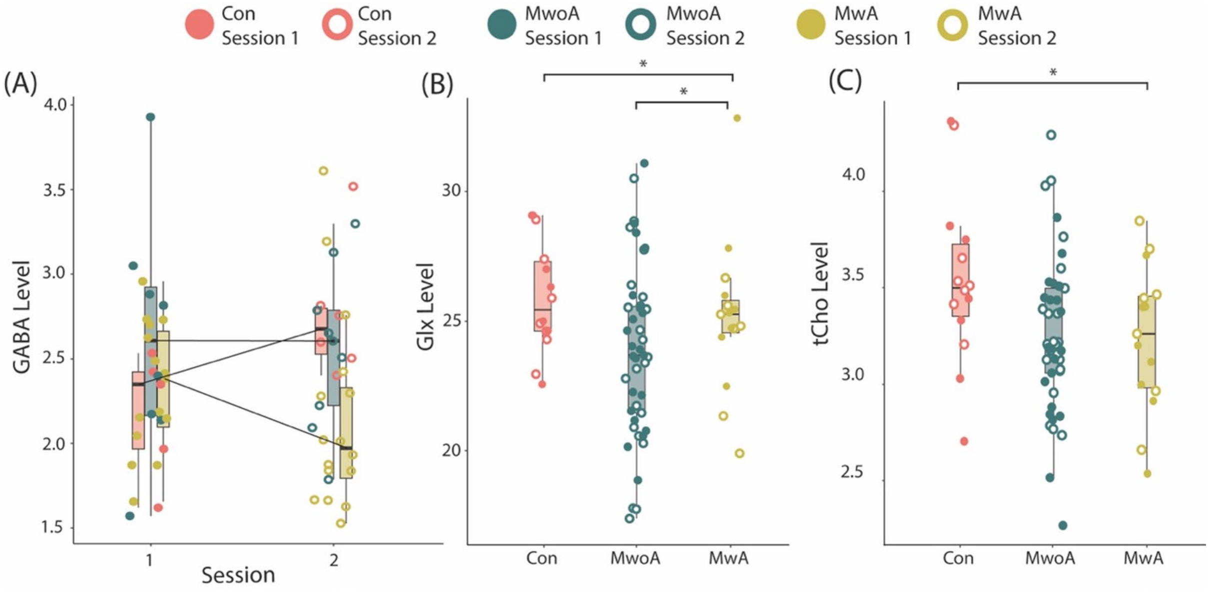
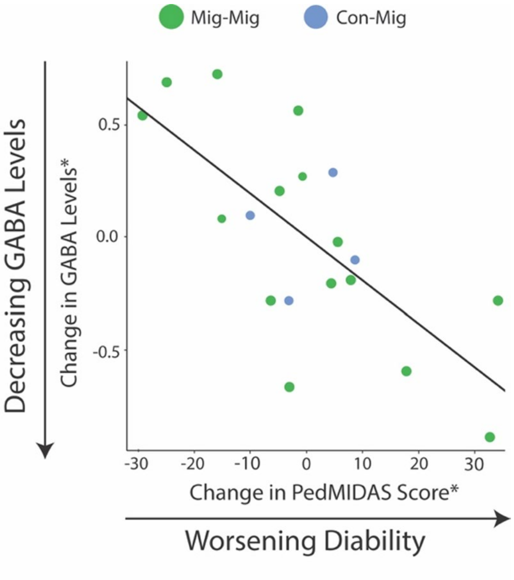


Fig. 5. Sensorimotor cortex metabolite levels across time and aura status. (**A**) Significant group by timepoint interaction on GABA levels in the sensorimotor cortex (F(2,59) = 3.25, p = 0.04, p_B = 0.12). Pairwise comparisons showed a non-significant difference between timepoint 1 and 2 in the migraine with aura group (t(42.7)=−0.47, p = 0.08). (**B**) Significantly lower sensorimotor Glx levels in the migraine with aura group compared to controls (t(34.0) = 2.41, p = 0.05), and in the migraine without aura group compared to migraine with aura (t(33.9)=−2.46, p = 0.05) (**C**) Significantly lower sensorimotor tCho levels in the migraine with aura group compared to controls (t(34.5) = 2.54, p = 0.04).

Sensorimotor cortex

GABA levels in the sensorimotor cortex showed a significant aura status by timepoint interaction, although this did not survive correction for multiple comparisons (F(2,59) = 3.25, p = 0.04, p_B = 0.12). Pairwise comparisons showed a non-significant decrease between timepoint 1 and 2 in the migraine with aura group only (t(42.7)=−0.47, p = 0.08; Fig. 5A).

Glx levels in the sensorimotor cortex showed a significant effect of aura status, although this did not survive correction for multiple comparisons (F(2,34.2) = 3.88, p = 0.03, p_B = 0.09). Pairwise comparisons showed Glx levels were significantly lower in migraine with aura compared to controls (t(34.0) = 2.41, p = 0.05) and lower in migraine without aura compared to migraine with aura (t(33.9)=−2.46, p = 0.05; Fig. 5B).



*Residuals from partial regression

Fig. 6. Significant relationship between the change in PedMIDAS score and the change in GABA levels in the thalamus ($b=-0.02$, $SE=0.03$, $p<0.001$, $p_B<0.001$).

tCho levels in the sensorimotor cortex also showed a significant effect of aura status, although this did not survive correction for multiple comparisons ($F(2,35.0)=3.25$, $p=0.05$, $p_B=0.15$). Pairwise comparisons showed tCho levels were significantly lower in migraine with aura compared to controls ($t(34.5)=2.54$, $p=0.04$; Fig. 5C).

There were no other significant differences in neurochemical levels in the sensorimotor cortex between participants with and without aura.

Occipital cortex

tCho levels in the occipital cortex showed a significant effect of aura status, although this did not survive correction for multiple comparisons ($F(2,25.9)=4.02$, $p=0.03$, $p_B=0.09$). Pairwise comparisons showed tCho levels were significantly lower in migraine with aura compared to controls ($t(24.0)=2.80$, $p=0.03$).

There were no other significant changes in occipital metabolite levels when separating by aura status, though of the nine participants who had aura, only four had GABA measurements from the occipital cortex.

Associations between changes in neurochemical levels and migraine disability

There was a significant relationship between the change in PedMIDAS score and the change in GABA levels in the thalamus ($b=-0.01$, $SE=0.003$, $p<0.001$, $p_B<0.001$; Fig. 6), as well as significant effects of group ($b=-1.11$, $SE=0.21$, $p=0.001$, $p_B=0.003$), months between scans ($b=0.05$, $SE=0.01$, $p<0.001$, $p_B<0.003$), and sex ($b=0.76$, $SE=0.20$, $p=0.002$, $p_B=0.006$). As seen above, the Con-Mig group had a larger decrease in GABA compared to the Mig-Mig group ($t(15)=1.13$, $p=0.001$). There was a larger increase in GABA levels in those with a longer amount of time between scans, and boys demonstrated a larger decrease in GABA levels compared to girls.

There were no significant relationships between the change in PedMIDAS score and the change in any other metabolite levels or brain areas that were measured. Additionally, there were no significant relationships between the change in headache days per month and the change in any of the metabolite levels in any of the brain areas.

Discussion

In this longitudinal study, we assessed changes in neurochemical levels and their relationship with migraine progression during adolescence, the first study of its kind. We demonstrate that thalamic GABA levels decrease over time with both new onset migraine and worsening of migraine disability. We also find group differences in tCr and tCho levels; participants who developed migraine and those initially diagnosed with migraine both had lower levels compared to controls.

Thalamic GABA levels decreased over time in adolescents who were initially unaffected and developed migraine between the two visits. Furthermore, adolescents with migraine at their initial visit who showed increased disability over time (measured using the PedMIDAS) had larger decreases in thalamic GABA levels. There were no significant changes in glutamate levels over time in any of the groups in any brain region. A systematic review showed that adults with migraine generally have higher glutamate and higher GABA levels in various regions[29], though the majority of studies have been conducted in cortical regions. The authors postulated that one reason for this increase in GABA is to compensate for increased glutamate. As we saw changes in GABA but not glutamate over time, it appears that GABA changes in the thalamus may precede glutamate increases in other brain areas such as the sensorimotor and occipital cortices. GABAergic neurons in the thalamic reticular nucleus provide recurrent inhibition on thalamocortical relay cells in the dorsal thalamic nuclei, which influences cortical excitability[30]. A reduction in inhibition in the thalamus would therefore reduce inhibitory control of the thalamocortical relay cells, resulting in an increase in cortical excitability. Lower thalamic GABA levels may trigger migraine attacks in the early years, resulting in chronically higher cortical glutamate levels later in life, perpetuating the migraine cycle throughout adulthood. In line with this theory, familial hemiplegic migraine type 2 (FHM2) mice, who are genetically predisposed to migraine, have been shown to have lower GABA and higher glutamate levels in the thalamus compared to wild-type mice[31].

Interestingly we found lower glutamate levels in the occipital cortex of youth with migraine with aura compared to controls. This is in line with our previous findings[11], but potentially contradicts the previously mentioned cortical hyperexcitability hypothesis[3]. We also found sensorimotor GABA levels decreased over time in youth with migraine with aura which was not seen in controls or youth with migraine without aura, but is consistent with cortical hyperexcitability. Previous MRS studies of the occipital cortex in adults with migraine have generally shown no difference in GABA levels[32–34] (although Bridge et al. found lower occipital cortex GABA levels in adults with migraine with aura compared to controls[35]), and an increase in glutamate[10] levels, the opposite direction to the results from the present study. This difference may be due to the lower age studied here, highlighting the need to study pediatric migraine specifically, rather than relying on findings from adult data. Further, although MRS measured glutamate is often interpreted as a measure of excitability, glutamate has other roles in the brain, including energy metabolism through the citric acid cycle[36]. The lower glutamate seen in the present study may therefore not reflect lower excitability, but instead an ongoing energy shortage, perhaps due to the hyperresponsiveness of the occipital cortex typically seen in migraine with aura[37]. Further research is needed to explore this finding.

The aim of this study was to scan participants interictally, but this was not always the case as migraine attacks are difficult to predict. Seven youth reported a headache on the day of the MRI scan, 6 reported one within 24 h, and 4 reported one within 48 h. It is worth noting that thalamic GABA levels have been shown to change over time with respect to migraine cycle onset. Previous studies have shown that adolescents who were closer to their next migraine attack had lower thalamic GABA[11] and thalamic GABA decreases over time as participants approach an attack[12]. While our results suggest increasing migraine prevalence and burden with decreasing GABA, it is possible that those who have more severe migraine burden also have shorter times between attacks. Therefore, there was a higher chance of scanning them during this preictal period (rather than a true interictal period) when thalamic GABA levels are lower. However, given that we didn't see relationships between migraine burden and GABA and glutamate in the sensorimotor and occipital cortex (previously shown to change with the migraine cycle[12], and proximity to attack had no effect on tCho and tCr levels previously[12], we can be more confident that our findings represent trait differences as opposed to state fluctuations.

We saw lower thalamic tCr levels in the Mig-Mig group compared to the Con-Con group. tCr is a combination of signal from creatine and phosphocreatine, both of which play a pivotal role in brain energy homeostasis[38]. Using phosphorus MRS, Lodi et al. found reduced PCr levels and higher ADP concentrations in youth with migraine compared to healthy controls, along with a higher rate of metabolism and a reduction in free energy availability[39]. Studies in adults with migraine have also shown similar findings of reduced energy potential, which could be indicative of a reduced mitochondrial reserve[40].

Interestingly, the Con-Mig group had lower tCho levels compared to controls across both timepoints, similar to the Mig-Mig group, suggesting this may be a trait of migraine across child development. tCho levels were also lower in the sensorimotor and occipital cortices of participants with migraine with aura compared to controls. Previously seen reductions in tCho levels in adults with migraine were interpreted to reflect alterations in membrane composition[41]. Alternatively, choline levels may be a marker of acetylcholine function[42]. Acetylcholine acts as an anti-inflammatory agent by suppressing glial activation. An animal model of chronic migraine demonstrated a decrease in nicotinic acetylcholine receptors and an increase in inflammatory astrocytes and proteins[43]. Acetylcholine also affects cortical excitability by increasing the threshold of cortical spreading depression initiation and decreasing propagation speed[44]. Based on this, it is possible that lower tCho levels may reflect a susceptibility to migraine, either due to natural reasons such as genetics, or due to repeated migraine attacks.

tNAA in the sensorimotor cortex decreased over time across all groups (control and migraine), consistent with previous research showing tNAA steadily decreases after age 10 in typical development[45–47]. As changes in NAA are related to changes in neuronal density and myelin synthesis and degradation[38], this decrease is likely due to synaptic pruning during typical brain development. Studies of healthy development have also shown relationships between age and Glx, tCr, and tCho[48]. It could therefore have been expected that we would see changes in these metabolite levels over time within our control group (and potentially in the migraine groups). However, these effects were detected using larger age ranges than covered here, and were previously not detected when using a smaller age range similar to that covered in this study[49]. It is likely that changes in these metabolites cannot be detected in a relatively short period due to the slow rate of change. The fact that GABA changes in the thalamus were seen during this shorter period in children who developed migraine provides support that these changes are migraine-dependent, rather than age-related. Additionally, age related changes are likely to be region-dependent. The sensory regions studied here are among the first to develop, and therefore undergo fewer changes in the adolescent period than association cortices[50], which are the more common locations for voxels measuring metabolite changes with age.

A strength of this study is the longitudinal design, providing unique insight into the relationship between neurochemical changes and migraine characteristics. To our knowledge, there are no other studies that have investigated neurochemical changes during adolescence and the relationship to migraine burden. Therefore, though our results are preliminary in nature, they provide unique knowledge about migraine progression and can guide future studies of this nature. Given the lack of effective treatments for youth with migraine and the fact that a younger age of onset is associated with a worse outcome later in life[51], research regarding the pathophysiology behind migraine progression during childhood/adolescence is key to identify early intervention opportunities.

Another strength of this study is the use of a macromolecule-suppressed GABA-editing sequence. The typically used GABA-editing acquisition results in contamination of the GABA signal with macromolecule signal (denoted as GABA+)[52]. Inclusion of this macromolecule signal has been shown to affect relationships between GABA and behavioural measures[53]. Additionally, Bell et al. did not find a previously reported relationship between GABA + and age[54] when using a macromolecule-suppressed sequence, implying macromolecule levels may be the driving force in this relationship[55]. The use of a macromolecule-suppressed GABA-editing sequence in this study ensures that the conclusions drawn are specific to GABA and not affected by changing macromolecule levels.

A limitation of this study is the small sample size per group. Recruitment for this study was of an opportunistic nature; participants from previous studies were invited back for a second MRI session. In some cases, the follow up time was as long as 5 years. Thus, in addition to typical study attrition, during this time some participants had moved away, for example to attend University. This resulted in a small sample size and may be biased as to who returned to participate. 26% of the possible migraine group participants and 38% of the control group participated in the second session. Additionally, half of our returning control group developed migraine between the two study visits. This does not reflect typical prevalence of migraine development in this age group and it is likely that controls who developed migraine were more inclined to participate in the second study visit due to the onset of migraine. There may also be additional unidentified biases in terms of participants who returned for the second timepoint. It should also be noted that the time between visits was shorter for the Mig-Mig group than the Con-Mig and Con-Con groups. Participants were recruited from two studies, however only one of the studies had a control sample. This study occurred prior to the second study, leading to a longer time between visits for participants recruited from the control sample. Subsequently, time between visits/age at visit was included in all statistical models to control for this. Future prospectively designed longitudinal studies should include a longitudinal aspect from the outset to increase retention and balance the time between study visits between groups.

The two studies used for recruitment also had different voxel sizes for occipital cortex data. At the second timepoint we matched data collection (including voxel size) to timepoint 1. Due to our small sample size we didn't separate analyses by voxel size but pooled the data. While the larger voxel would include more tissue and would have differences in data quality (SNR and linewidth), given our statistical analysis was of a paired nature (comparing differences between timepoint 1 and timepoint 2), this is unlikely to have an effect on our results. Indeed, removal of participants with the smaller voxel from analyses did not change the findings.

This study provides preliminary results indicating neurometabolites change in relation to migraine across development. Given the physiological changes that occur during this period due to puberty, the strong sex differences in migraine epidemiology and symptomatology in adults[56], and that migraine prevalence sharply increases in females following puberty[57], a key question that follows from this study is the effects of sex and puberty on these neurometabolite changes. For example, connectivity differences in children with migraine compared to controls have been shown to be modulated by age, sex and pubertal status[58]. This is a key question and the mechanisms surrounding these effects are poorly understood. Although this study was not powered to assess these effects, sex was included as a covariate in all our analyses, and we did see a larger decrease in thalamic GABA levels in boys with migraine compared to girls with migraine. Previous work has shown reduced resting state functional connectivity between the thalamus and other brain areas in adolescent males with migraine compared to females[59]. Wierenga et al. (2018) showed decreases in thalamus volume in adolescent males were associated with pubertal status and testosterone changes, with males with higher levels of testosterone showing faster volume decreases. However no such relationship was seen in females[60]. Therefore, sex specific effects of brain maturation may contribute to sex specific effects seen in the migraine brain, highlighting the importance of further studying these effects. The results presented here can be used to guide future longitudinal studies of migraine across the adolescent period with a study sample size large enough to consider effects related to pubertal status and disaggregation by sex.

While these data are preliminary and need to be interpreted with caution due to the above mentioned limitations, these data provide some preliminary directions for approaches to mitigate migraine across development. For example, GABA in the thalamus is expected to increase with typical development while it is unchanged in patients with migraine and it lowers in participants who developed migraine. Consistent with this group analysis, greater reductions in thalamic GABA were associated with increasing migraine disability. Therefore, supporting healthy GABA increases across development may be a means to mitigate the effects of migraine. Conversely, the lower choline levels in the thalamus and occipital cortex of children with migraine at both visits and those who developed migraine suggests choline alterations exist prior to the onset of migraine and may be a potential biomarker of migraine susceptibility. Inflammation may be an additional treatment target regardless of development.

In conclusion, we show that a decrease in thalamic GABA levels over time is related to new onset migraine in previously unaffected adolescents and increased disability in those with migraine. This provides new evidence for a role of thalamic GABA early in migraine incidence and progression, and a potential opportunity for new treatment targets.

Data availability

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

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Author contributions

TB: methodology, formal analysis, investigation, writing – original draft, writing – review and editing, visualization, project administration. LC: investigation, writing – review and editing. JK: investigation, writing – review and editing. KM: investigation, writing – review and editing. ADH: funding acquisition, methodology, writing – review and editing. SLO: funding acquisition, writing – review and editing, ADH: conceptualization, methodology, funding acquisition, writing – review and editing, supervision.

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Declarations

Competing interests

The authors declare no competing interests.

Additional information

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