Supplementary materials

Supplementary table 1. Review of studies of investigating the volume of the Hb

Study	Sample Size	Sequence; FOV; matrix; voxel size; TR/TE (ms); AT	B0; vendor; coil	Key results
Savitz et al., 2011	PTSD (11) vs. HC (75)	MPRAGE; 14 cm; 224×224; 0.55×0.55×0.6 mm; 7.8/2.1; Three to four 13-minute scans A second MPRAGE for whole brain; Not Mentioned; Not Mentioned; 0.85 × 0.85 × 1.2 mm; 11.6/4.94; Not Mentioned	3.0T; GE; head radiofrequency coil	did not differ significantly in absolute or normalized habenula volume
Savitz et al., 2011	unmedicated, depressed MDD and unmedicated MDD in remission (28 and 32), medicated and unmedicated BD (15 and 22) vs. HC (74)	MPRAGE; 14 cm; 224×224; 0.55×0.55×0.6 mm; 7.8/2.1; Three to four 13-minute scans A second MPRAGE for whole brain; Not Mentioned; Not Mentioned; 0.85 × 0.85 × 1.2 mm; 11.6 /4.94; Not Mentioned	3.0T; GE; head radiofrequency coil	1.Unmedicated BD patients displayed significantly smaller absolute and normalized Hb volumes than the HCs. 2. Women with MDD had smaller absolute Hb volumes than the HC women
Carceller et al., 2015	first-episode MDD, remitted-recurrent MDD and treatment-resistant/ chronic MDD (21,20 and 20) vs. HCs (34)	MPRAGE; 256×256×204; 256×240; 0.89×0.89×1.2 mm; 6.7/3.2; Not Mentioned	3.0T; Philips; head coil	Women with a first-episode MDD had greater Hb-white matter volumes than HCs and patients with treatment-resistant/chronic MDD
Bocchetta et al., 2016	FTD，AD (15,15) vs. HCs (15)	Volumetric T1WI; Not Mentioned; 256×256; 1.1 mm; 2200/2.9; Not Mentioned	3.0T; Siemens; head coil	1. FTD group showed a lower Hb volume compared with HCs 2. AD group was not significantly different to HCs 3. FTD group was significantly smaller than AD
Schmidt et al., 2017	unmedicated and medicated MDD (20 and 20) vs. HCs (20)	MP2RAGE-T1 mapping; 224×224; 320×320; 0.7×0.7×0.7 mm; 8250/2.51; 18:02 min	7.0T; Siemens; head coil	1. Absolute and relative Hb volumes did not differ significantly among the three groups 2. Significant correlations were found between Hb volumes and HAMD-17- and BDI-II-related severities.
Zhang L et al., 2017	SCH (15) vs. HCs (16)	SPGR; 256×256; 256×256; 1×1×1 mm; 8.2/3.2; Not Mentioned	3.0T; GE; head radiofrequency coil	Absolute Hb volume was significantly lower in the SCH than in the HCs.
Luan SX et al., 2019	TRD (15) vs. HCs (15)	SPGR; 256×256; 256×256; 1×1×1 mm; 8.2/3.2; Not Mentioned	3.0T; GE; head radiofrequency coil	No significant difference of Hb absolute volume between TRD patients and HCs
Cho S-E et al., 2021	MDD (33) vs. HC (36)	MP2RAGE; 166×166×135.2; 256×256; 0.65×0.65×0.65 mm; 8000/3.46, 7.28, 11.1, 14.92; 14:16 min	7.0T; Siemens; head coil	MDD patients had a smaller right Hb volume

FOV: field of view; AT: acquire time; PTSD: post-traumatic stress disorder; HCs: healthy controls; FTD: frontotemporal dementia; AD: Alzheimer's disease; MDD: major depressive disorder; BD: bipolar disorder; SCH: schizophrenia; TRD: treatment-resistant depression

Supplementary table 2. Acquisition parameters of 3.0T MPRAGE, MP2RAGE and QSM sequences

	MPRAGE	MP2RAGE	QSM
Field of view	230×230 mm	230×230 mm	220×220 mm
Number of slices	208	208	128
Repetition time	1800.0 ms	4750.0 ms	50.0 ms
Echo time	2.41 ms	2.13 ms	7.73ms, 14.97 ms 22.21ms, 29.45 ms 36.69 ms, 43.93 ms
Inversion time	850 ms	700ms and 2500ms	-
Flip angle	8 deg.	4 deg. and 5 deg.	15 deg.
Voxel size (mm^3)	0.8×0.8×0.8	0.8×0.8×0.8	0.5×0.5×0.8
Readout bandwidth	200 Hz/Px	230 Hz/Px	220 Hz/Px
Parallel acquisition	GRAPPA, factor 2	GRAPPA, factor 2	GRAPPA, factor 2
Acquisition time	4:07	10:46	12:11

MPRAGE, magnetization-prepared rapid gradient echo; MP2RAGE, magnetization-prepared 2 rapid acquisition gradient echoes; QSM, quantitative susceptibility mapping.

Supplemental table 3. Intraclass correlation coefficient of SNR, CNR and T1 values of habenula by two radiologists from MP2RAGE-UNI and MPRAGE

ICC (95% confidence interval)										
Sequence	MP2RAGE-UNI						MPRAGE			
	Right Habenula			Left Habenula			Right Habenula		Left Habenula	
	SNR	CNR	T1 values	SNR	CNR	T1 values	SNR	CNR	SNR	CNR
First Scan	0.757 (0.270 to 0.934)	0.910 (0.595 to 0.978)	0.948 (0.812 to 0.987)	0.757 (0.270 to 0.934)	0.910 (0.595 to 0.978)	0.967 (0.881 to 0.992)	0.722 (0.170 to 0.925)	0.868 (0.558 to 0.965)	0.722 (0.170 to 0.925)	0.868 (0.558 to 0.965)
Second Scan	0.867 (0.576 to 0.965)	0.857 (0.545 to 0.962)	0.991 (0.961 to 0.998)	0.867 (0.576 to 0.965)	0.857 (0.545 to 0.962)	0.985 (0.994 to 0.971)	0.772 (0.336 to 0.938)	0.647 (0.126 to 0.896)	0.772 (0.336 to 0.938)	0.647 (0.126 to 0.896)
All $P < 0.05$; Note: bilaterally averaged SNR and CNR from UNI and MPRAGE										

Supplemental table 4. Manual segmentation volume of habenula in MP2RAGE-UNI and MPRAGE

		First Scan								Second Scan							
		MP2RAGE-UNI				MPRAGE				MP2RAGE-UNI				MPRAGE			
		Radio 1		Radio 2		Radio 1		Radio 2		Radio 1		Radio 2		Radio 1		Radio 2	
		first	second	first	second	first	second	first	second	first	second	first	second	first	second	first	second
1	L	33.1	33.2	32.7	31.5	33.2	34.5	31.7	29.5	32.0	32.3	33.7	31.5	34.5	35.0	30.7	29.5
	R	34.4	34.0	33.4	32.3	35.7	32.7	32.4	30.3	33.4	33.0	32.4	32.6	35.2	32.7	31.3	31.2
2	L	33.5	34.8	32.7	33.2	34.7	37.8	30.7	33.7	33.5	33.7	32.4	33.3	33.7	33.3	30.3	34.4
	R	32.7	34.3	31.3	32.8	32.7	36.2	30.2	33.8	33.7	34.2	31.8	32.7	33.5	34.2	30.6	33.6
3	L	32.0	33.2	32.0	33.3	32.7	39.3	32.0	36.5	32.0	32.2	32.5	32.7	30.4	36.3	33.0	34.6
	R	32.3	33.2	33.4	34.6	32.7	37.8	34.3	35.6	32.4	33.5	33.4	33.6	32.2	37.8	34.3	34.7
4	L	33.5	34.4	32.7	34.1	32.7	34.7	33.7	36.5	33.6	34.5	32.5	33.1	32.5	35.7	34.7	34.5
	R	30.1	32.2	31.8	32.1	30.1	36.2	31.8	37.2	31.1	31.6	33.6	32.0	31.7	34.2	30.9	35.2
5	L	29.6	30.0	30.6	29.0	29.6	24.0	30.6	26.0	29.7	30.0	30.3	30.0	29.1	29.0	31.8	29.0
	R	31.6	29.5	29.6	29.6	31.6	27.5	28.6	29.6	31.3	30.5	28.6	29.2	33.4	29.5	29.6	32.5
6	L	30.7	30.1	29.1	30.7	30.1	25.5	29.1	27.7	31.8	30.9	29.2	30.2	30.2	28.6	29.9	28.6
	R	31.1	30.5	30.7	29.8	32.1	26.5	34.7	29.2	31.2	30.9	30.4	29.9	31.2	28.7	34.8	30.2
7	L	36.5	36.2	35.7	34.8	37.2	36.2	34.7	33.8	36.3	35.1	35.7	35.8	34.5	34.2	31.7	33.5
	R	38.3	37.7	36.8	36.6	39.8	37.8	36.8	36.7	38.1	37.1	36.5	35.6	38.6	37.4	35.8	34.7
8	L	27.9	28.3	28.0	27.6	26.0	28.6	24.0	28.6	28.9	28.7	28.2	27.8	26.2	27.6	24.7	30.6
	R	24.0	24.3	25.0	25.2	24.0	24.5	26.0	25.5	24.0	24.4	25.0	25.3	24.0	26.0	27.0	26.5
9	L	37.3	37.0	35.3	36.7	38.3	32.7	33.3	30.7	36.5	36.0	35.8	36.5	35.3	33.5	33.3	32.7
	R	38.8	36.7	36.8	35.4	39.8	33.2	34.8	34.4	37.8	35.7	36.5	35.5	34.9	34.6	32.9	35.4
1	L	34.	35.2	33.	34.2	34.	36.2	36.	34.7	35.	35.4	33.	34.5	33.	35.5	36.	33.4

0		7		7		7		7		6		8		9		7	
	R	33.7	34.0	34.6	35.1	33.6	34.1	36.6	35.1	34.9	33.7	34.5	35.3	32.6	33.7	35.7	33.2
Unit: mm^3																	

Supplemental table 5. Measured T1 Values from First and Second MP2RAGE Scan

No./Sex/Age	First Scan				Second Scan			
	Radio 1		Radio 2		Radio 1		Radio 2	
	Right Hb	Left Hb	Right Hb	Left Hb	Right Hb	Left Hb	Right Hb	Left Hb
1/M/25	940	924	932	932	929	924	933	927
2/M/31	860	971	852	971	860	989	853	987
3/M/40	944	935	940	941	970	886	961	889
4/M/28	927	923	928	926	934	911	934	907
5/M/33	951	947	945	940	934	954	926	962
6/F/32	909	884	904	866	888	887	886	886
7/F/38	1009	993	1004	991	987	989	994	971
8/F/27	968	987	986	986	988	1005	988	1005
9/F/30	880	947	838	937	838	936	832	927
10/F/31	944	948	938	935	932	964	918	959
Mean ± SD	933.2±42.6	945.9±32.4	926.7±51.7	942.5±35.5	926.0±50.7	944.5±42.7	922.5±53.2	942.0±41.0
COV (%)	4.6	3.4	5.6	3.8	5.5	4.5	5.8	4.4
Paired t-test	0.377		0.350		0.353		0.335	

Radio = radiologist; COV = coefficient of variation; MAD = median absolute difference; MP2RAGE, magnetization-prepared 2 rapid acquisition gradient echoes

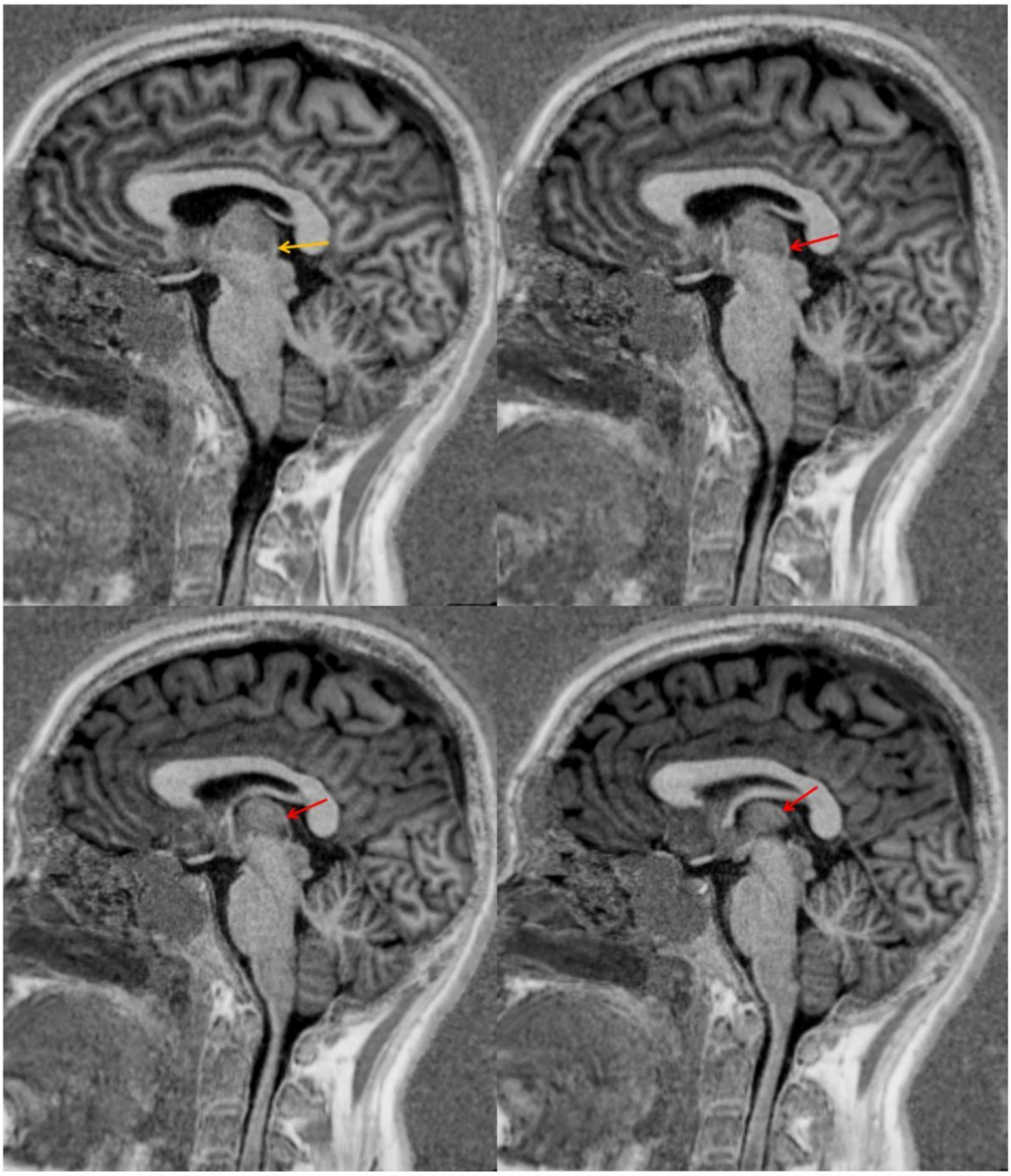

Figure 1. In MP2RAGE-UNI, the habenula is hyperintense compared to the thalamus (yellow arrow). Stria medullaris formed aarch that curves upward into the habenula also is hyperintense due to relatively dense white matter fibers (red arrow).

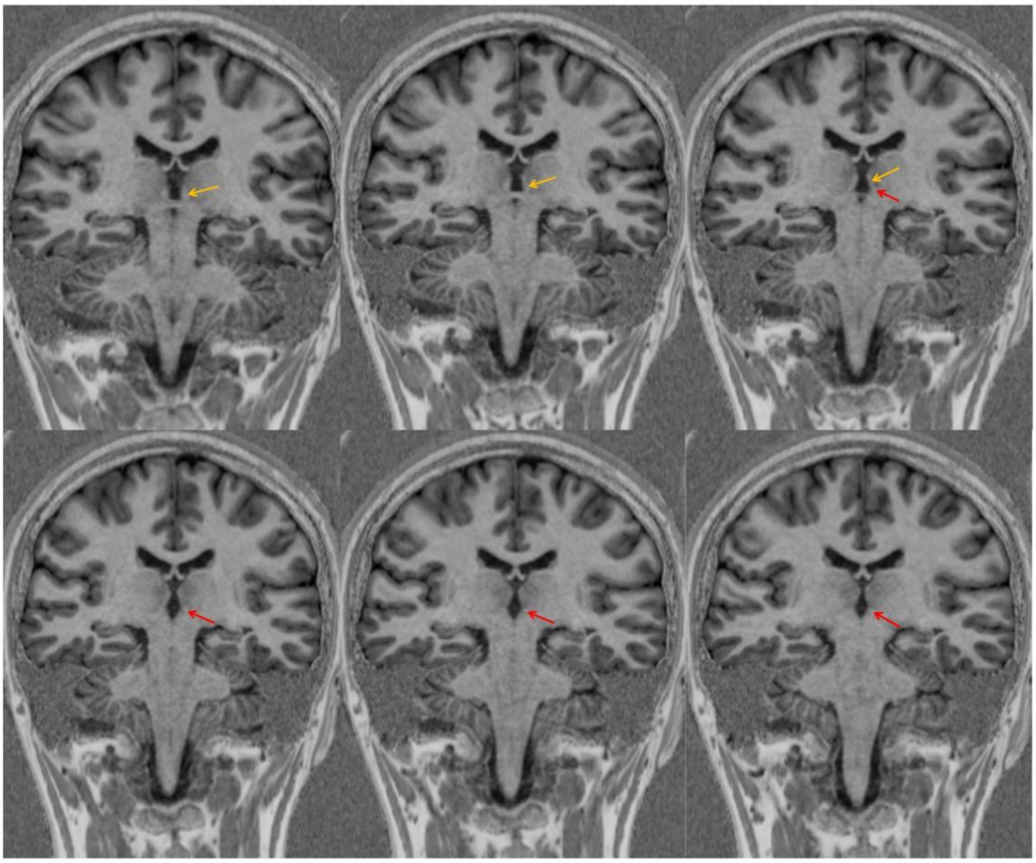

Figure 2. In MP2RAGE-UNI, the habenula is hyperintense compared to the thalamus (yellow arrow). Fasciculus retroflexus is lightly hyperintense due to relatively dense white matter fibers (red arrow).

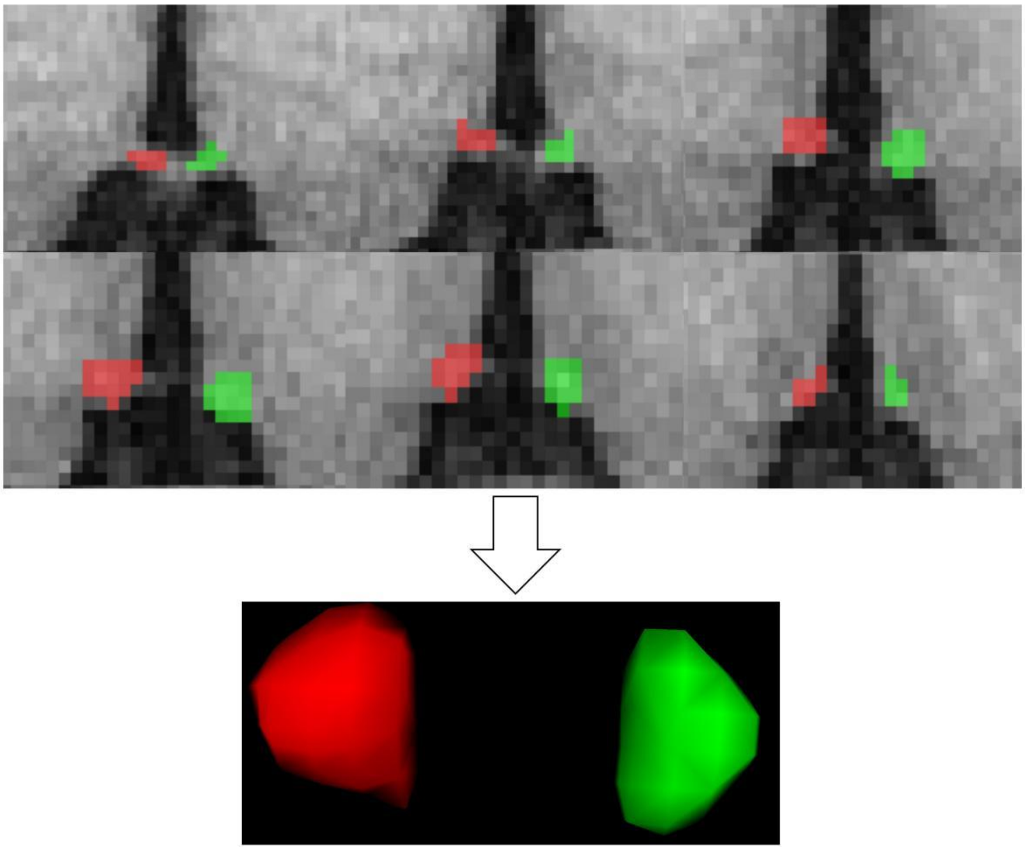

Figure 3. An example of the 3D volume reconstruction for the habenula volume calculation by ITK SNAP software in MP2RAGE-UNI sequence. The Hb boundaries were traced from the slice above the posterior commissure for five to six or seven slices until the Hb was no longer visible.

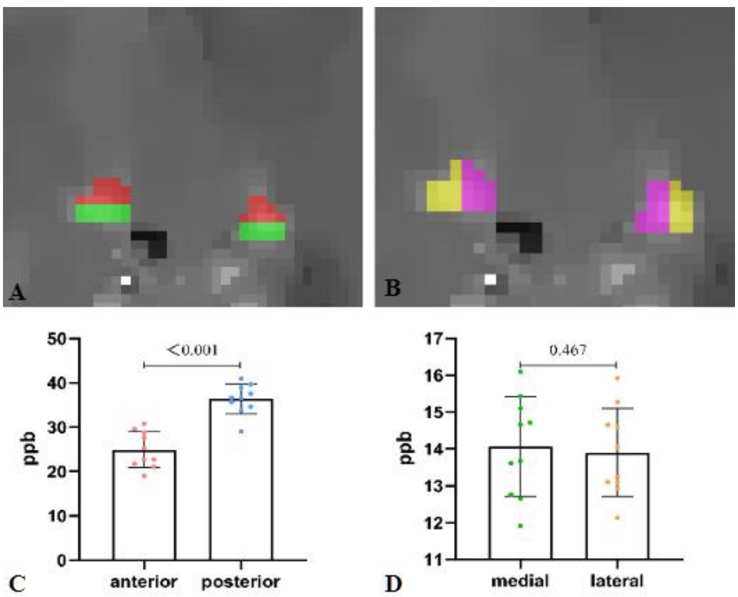

Figure 4. Regions of interest on the quantitative susceptibility mapping from the left to right habenula of a representative

participant. (A) Anterior (red) and posterior (green) subregions within the habenula. (B) Medial (pink) and lateral (yellow) subregions within the habenula. (C) and (D) The susceptibility of the posterior halves of the habenula are higher than the anterior halves, and the medial halves are higher than the lateral halves.

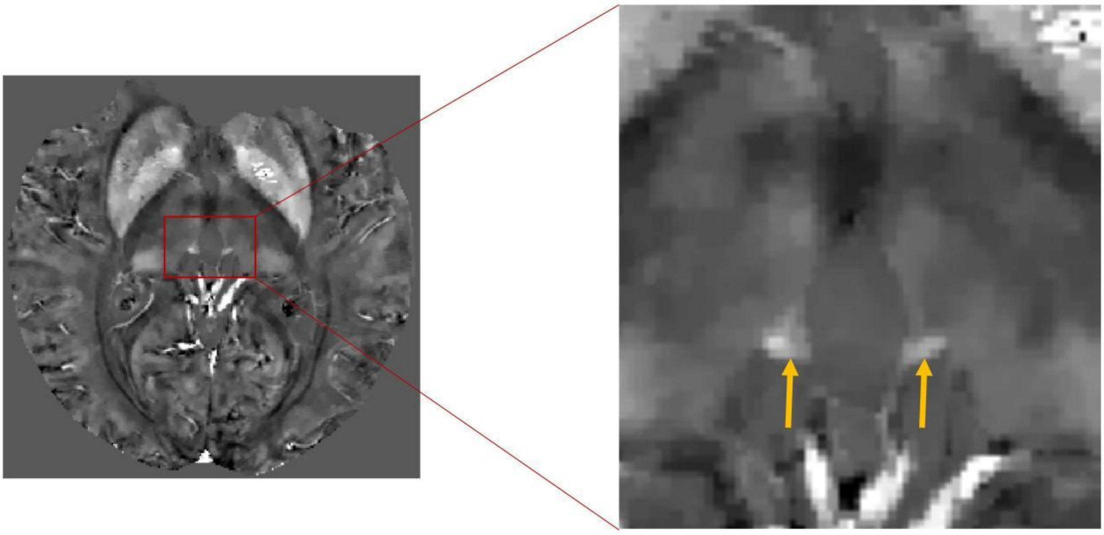

Figure 5. Example of quantitative susceptibility mapping around the habenula (yellow arrows) on an axial slice.

QSM data were reconstructed for six echo individually by an in-house algorithm. The concrete steps included: 1) isolate the brain tissue using the FSL Brain Extraction Tool (http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/BET) (threshold = 0.2, erode = 4, and island = 2000); 2) a 3D phase unwrapping algorithm (3DSRNCP) was used to unwrap the original phase data; 3) remove unwanted background fields by the sophisticated harmonic artifact reduction (SHARP) filter (threshold= 0.05 and deconvolution kernel size = 6); 4) A truncated k-space division-based inverse filtering technique (threshold = 0.1) with an iterative approach (iteration threshold = 0.1 and number of iterations = 4) was used to reconstruct the susceptibility map.

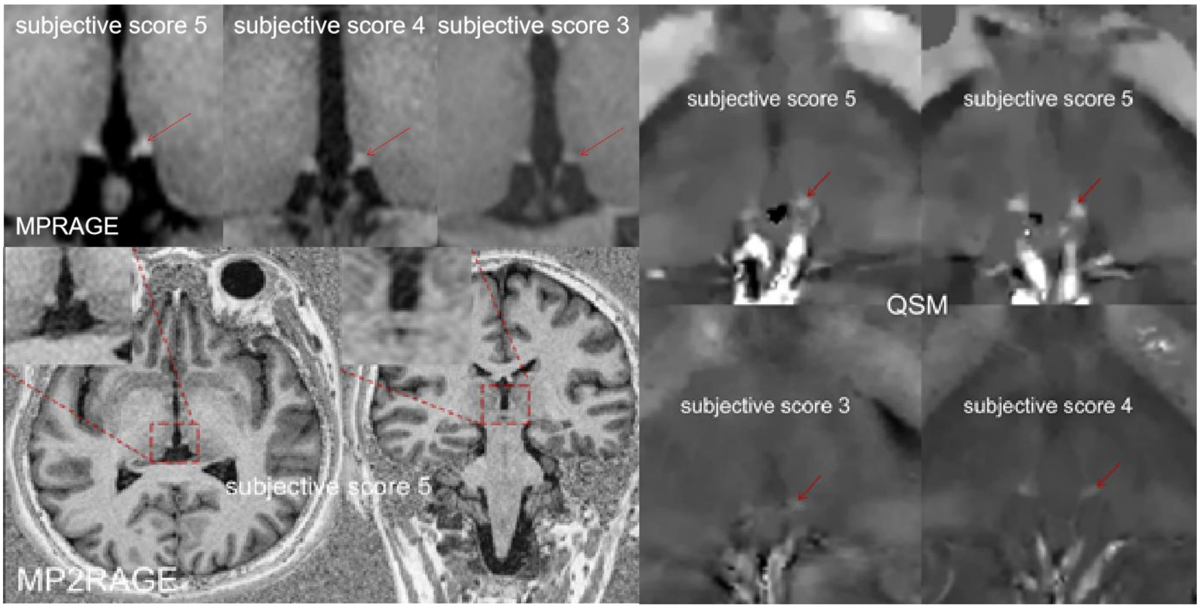

Figure 6. Representative subjective scores of MPRAGE, MP2RAGE and QSM of the habenula. MPRAGE, magnetization-prepared rapid gradient echo; MP2RAGE, magnetization-prepared 2 rapid acquisition gradient echoes; QSM, quantitative susceptibility mapping.