Longitudinal study of hippocampal volumes in heavy cannabis users

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Supplementary material

1. Detailed manual tracing protocol for the hippocampus

Hippocampi were manually traced via the software ANALYZE (Mayo Clinic, Rochester, NY; http://mayo.edu/bir/). Tracing proceeded from the caudal (i.e. hippocampal tail) to the rostral end of the brain (i.e. hippocampal head), on coronally displayed brain slices. Volumetric estimates were obtained by summing all voxels within the traced hippocampus on consecutive coronal slices. To facilitate tracings speed and encourage focus on the area of interest, image visualization was cropped around the medial temporal lobe. Tracings were copied from slice to slice and edited according to changes of hippocampal boundaries through each slice, which proceeded differently for the left and right hemisphere. Copying tracings to the following slices promoted consistency between slices and facilitated tracing where hippocampal boundaries were less visible, since image artifacts caused by the loss of MRI signal that occurs in the medial temporal region and in vicinity to sinuses can compromise visualization of the hippocampus. Hippocampal tracings included the hippocampus proper, dentate gyrus and part of the fimbria and alveus (Velakoulis, Pantelis, McGorry, & al, 1999; Velakoulis, Wood, Wong, & al, 2006).

Adapted from Lorenzetti, 2012. The impact of regular cannabis use on human brain structure. Personal communication.

Supplementary table 1. Output of the repeated measures Analysis of Covariance test, both within-subjects and between-subjects contrasts.

	F	*p*	Partial Eta Squared
BETWEEN SUBJECTS			
gender	10.331	0.003	0.21
age	0.001	0.976	0.00
group	0.167	0.685	0.00
WITHIN SUBJECTS			
time point	26.215	0.000	0.40
time point * gender	3.981	0.053	0.09
time point * age	3.272	0.078	0.08
time point * group	0.008	0.928	0.00
hemisphere	90.516	0.000	0.70
hemisphere * gender	0.017	0.898	0.00
hemisphere * age	0.070	0.793	0.00
hemisphere * group	0.785	0.381	0.02
time point * hemisphere	12.039	0.001	0.24
time point * hemisphere * gender	1.955	0.170	0.05
time point * hemisphere * age	0.455	0.504	0.01
time point * hemisphere * group	1.086	0.304	0.03

Supplementary table 2. Output of the repeated measures Analysis of Covariance test within the cannabis group. The hippocampal volumes (adjusted for intracranial volume) were the dependent variable. We used a median split of cumulative dosage at baseline, i.e. low cumulative dosage (N=9, < 351 cumulative grams) versus high cumulative dosage (N=10, > 351 cumulative grams) and entered age (age at baseline centered around the grand mean), age of onset of regular use, dosage change and gender as covariates.

	F	p	Partial Eta Squared
BETWEEN SUBJECT EFFECTS			
age	5.932	0.038	0.397
age onset regular use	3.723	0.086	0.293
dosage change	0.503	0.496	0.053
cumulative dosage at BL, median split	1.942	0.197	0.177
gender	0.320	0.586	0.034
WITHIN-SUBJECT EFFECTS			
time point	0.530	0.485	0.056
time point * age	1.564	0.243	0.148
time point * age onset regular use	0.143	0.714	0.016
time point * dosage change	1.184	0.305	0.116
time point * cumulative dosage at BL, median split	1.128	0.316	0.111
time point * gender	1.032	0.336	0.103
time point * cumulative dosage at BL, median split * gender	0.006	0.942	0.001
hemisphere	0.005	0.947	0.001
hemisphere * age	0.047	0.833	0.005
hemisphere * age onset regular use	0.318	0.587	0.034
hemisphere * dosage change	0.002	0.967	0.000
hemisphere * cumulative dosage at BL, median split	0.438	0.525	0.046
hemisphere * gender	0.017	0.900	0.002
hemisphere * cumulative dosage at BL, median split * gender	0.000	0.990	0.000
time point * hemisphere	0.255	0.625	0.028
time point * hemisphere * age	0.227	0.645	0.025
time point * hemisphere * age onset regular use	0.219	0.651	0.024
time point * hemisphere * dosage change	1.844	0.208	0.170
time point * hemisphere * cumulative dosage at BL, median split	0.637	0.445	0.066
time point * hemisphere * gender	0.475	0.508	0.050
time point * hemisphere * cumulative dosage at BL, median split * gender	0.742	0.411	0.076

Supplementary table 3. Post-hoc analyses showing the effect of time with significantly larger volumes on follow up than on baseline for both cannabis users and controls.

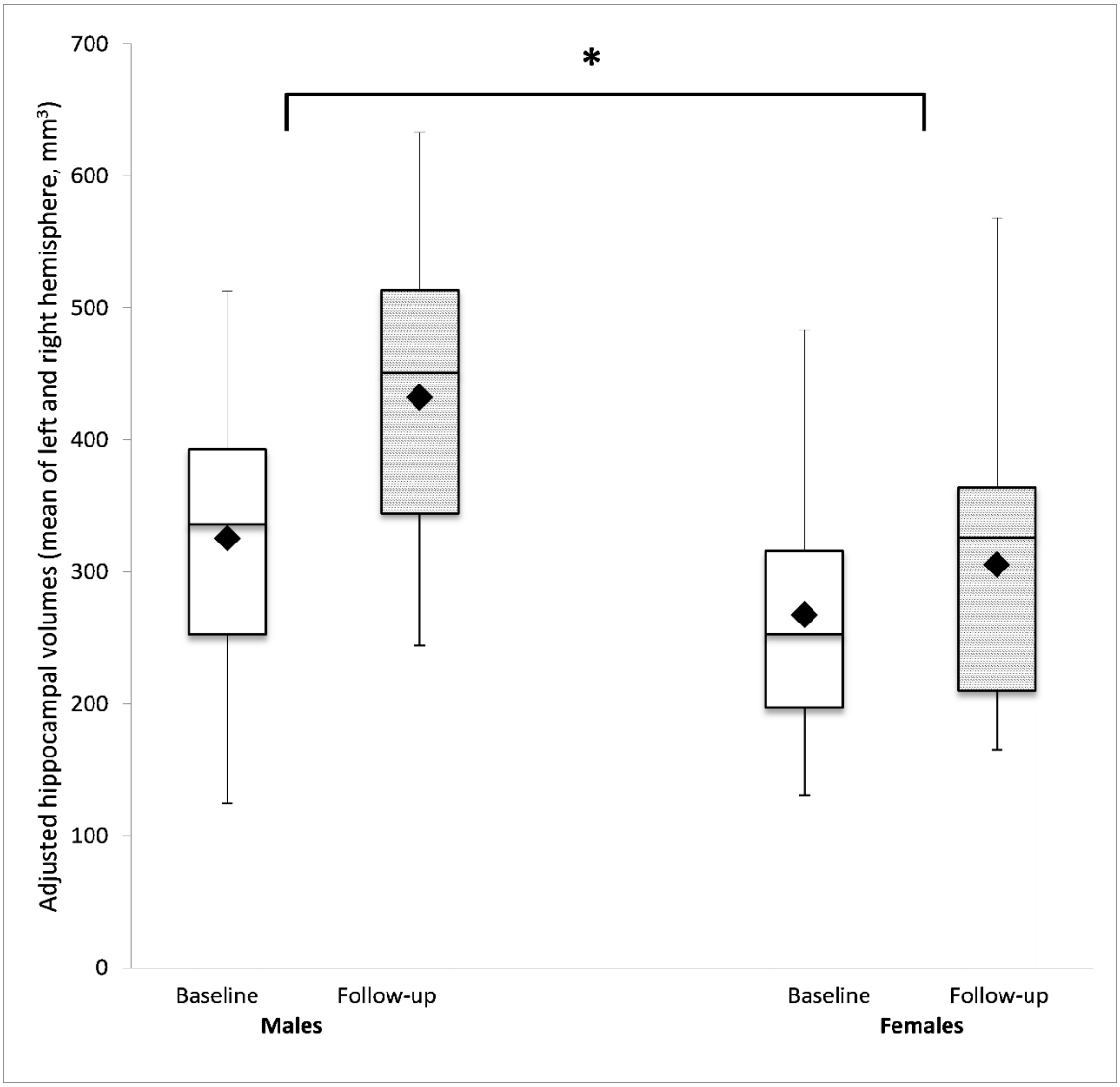
Group	**Time**	**Hippocampal volume**		***p***
		L&R hemi, M(SD)	*Mean Difference (BL-FU)*	*Difference BL vs FU*
CB	**BL**	298.45 (23.41)	-80.97	0.003
	FU	379.41 (25.92)		
HC	**BL**	308.93 (21.77)	-84.15	0.001
	FU	393,08 (24,10)		

Note: BL=baseline, FU=follow up, CB=cannabis, HC= healthy controls, M=mean, SD=standard deviation, L=left, R=right, hemi=hemisphere, p=p-value, significant from p<.05.

Supplementary table 4. Means and standard deviations of the corrected hippocampal volumes for males and females.

Gender	Time	Hemisphere	CB, M (SD)	HC, M (SD)
Males	**BL**	*L*	381 (137)	386 (100)
		R	260 (99)	281 (83)
	FU	*L*	487 (130)	451 (106)
		R	393 (114)	407 (142)
Females	**BL**	*L*	308 (143)	327 (107)
		R	205 (107)	222 (75)
	FU	*L*	321 (72)	364 (119)
		R	244 (78)	275 (129)

Note: BL=baseline, FU=follow up, CB=cannabis, HC= healthy controls, M=mean, SD=standard deviation, L=left, R=right.



Supplementary figure 1. Boxplot of the adjusted hippocampal volumes, grouped by gender and time point (mean values of hemisphere). Males have significantly larger hippocampal volumes than females. There was a non-significant trend for an interaction effect between time point and gender, indicating that the males increased slightly more than the females over time ($F(1)= 3,981$, $p=.053$, $\eta^2=.093$).

Supplementary table 5. Post-hoc analyses showing the lateralization effect with significantly larger volumes in the left than in the right hemisphere.

Time	**Group**	**hippocampal volume**		***p***	**hippocampal volume**			***p***
		L&R, M (SD)	*Mean Difference, both groups (L-R)*	*Difference BL vs FU*	*L hemi, M (SD)*	*R hemi, M (SD)*	*Mean Difference (L-R)*	*Difference L&R per time point per group*
BL	CB	298.45 (23.41)	-113.43	0.000	355.75 (27.55)	241.15 (20.75)	114.61	0.000
	HC	308.93 (21.77)			365.05 (25.62)	252.80 (19.30)	89.93	0.000
FU	CB	379.41 (25.92)	-76.63	0.000	424.38 (26.08)	334.45 (27.88)	112.25	0.000
	HC	393.08 (24.10)			424.75 (24.26)	361.41 (25.93)	63.34	0.000

Note: BL=baseline, FU=follow up, CB=cannabis, HC= healthy controls, M=mean, SD=standard deviation, L=left, R=right, hemi=hemisphere, p=p-value, significant from p<.05.

Supplementary table 6. Overview of comorbidities within the cannabis users and healthy control group. These were assessed at follow-up. Note that exclusion criteria at baseline were (among others) having used non-cannabinoid illicit drugs during the last year or on more than 100 occasions lifetime and a history of axis one psychiatric disorders. This means that these comorbidities were developed in the 3-year interval between our baseline and follow-up assessment.

	CB	**HC**
No comorbidities, *N*	14	21
Comorbidities present at follow up, *N*	1: dependence unknown substance	
	1: depressive disorder, ADHD	1: cocaine and amphetamine dependence
	1: psychotic disorders NOS	1: depressive disorder
	1: depressive disorder	
	1: ADHD, social phobia, amphetamine dependence	
	1: depressive disorder, panic disorder, obsessive compulsive disorder, ADHD, cocaine abuse, amphetamine abuse	

Note: CB=cannabis, HC= healthy controls, N=number of subjects

Supplementary table 7a and 7b. Group analyses without the subjects with polysubstance use and/or a comorbid psychiatric disorder.

Table 7a. Means and standard deviations from the adjusted hippocampal volumes without the subjects with polysubstance use and/or a comorbid psychiatric disorder.

Time	hemisphere	Group	Mean	SD	N
BL	L	*CB*	327	144	14
		HC	366	111	21
	R	*CB*	230	109	14
		HC	251	86	21
FU	L	*CB*	451	134	14
		HC	404	113	21
	R	*CB*	369	125	14
		HC	345	152	21

Note: BL=baseline, FU=follow up, CB=cannabis, HC=healthy controls, SD=standard deviation, N=number

Table 7b. Output of the repeated measures Analysis of Covariance test, both within-subjects and between-subjects contrasts without the subjects with polysubstance use and/or a comorbid psychiatric disorder.

	F	Sig.	Partial Eta Squared
BETWEEN SUBJECT EFFECTS			
Gender	14.609	0.001	0.32
age at BL	0.820	0.372	0.03
Group	0.093	0.762	0.00
WITHIN-SUBJECT EFFECTS			
timepoint	33.098	0.000	0.52
timepoint * Gender	3.613	0.067	0.10
timepoint * age at BL	1.821	0.187	0.06
timepoint * Group	1.749	0.196	0.05
hemisphere	66.673	0.000	0.68
hemisphere * Gender	0.090	0.766	0.00
hemisphere * age at BL	0.019	0.891	0.00
hemisphere * Group	0.011	0.916	0.00
timepoint * hemisphere	7.644	0.010	0.20
timepoint * hemisphere * Gender	1.052	0.313	0.03
timepoint * hemisphere * age at BL	0.016	0.899	0.00
timepoint * hemisphere * Group	2.109	0.157	0.06

Supplementary tables 8a and 8b. Cannabis pattern analyses without the subjects with polysubstance use and/or a comorbid psychiatric disorder.

Table 8a. Means and standard deviations from the adjusted hippocampal volumes without the subjects with polysubstance use and/or a comorbid psychiatric disorder.

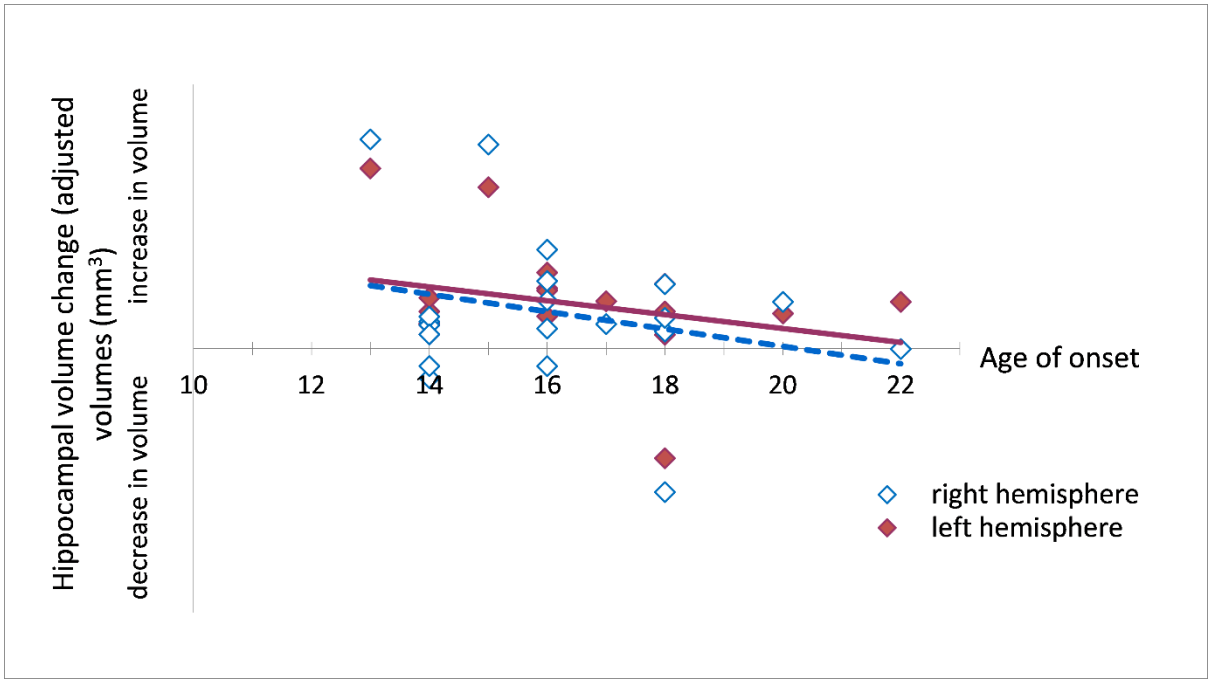
Time	hemisphere	cumulative dosage	Mean	SD	N
BL	L	*low*	345	141	6
		high	277	124	7
		total	309	131	13
	R	*low*	260	130	6
		high	183	66	7
		total	219	104	13
FU	L	*low*	450	140	6
		high	444	149	7
		total	447	139	13
	R	*low*	377	145	6
		high	352	123	7
		total	363	129	13

Note: BL=baseline, FU=follow up, L=left, R=right, SD=standard deviation, N=number

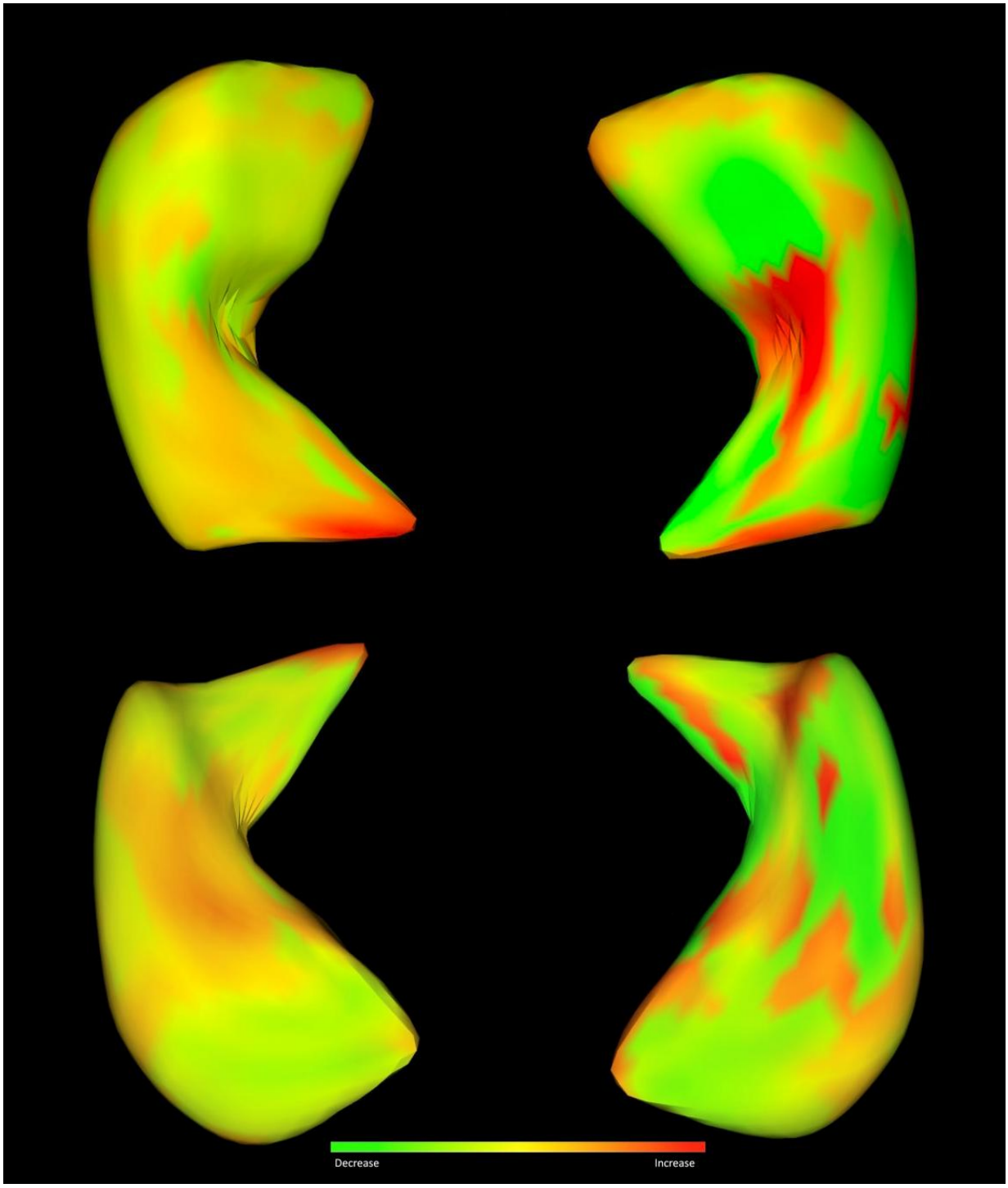
Table 8b. Output of the repeated measures Analysis of Covariance test, both within-subjects and between-subjects contrasts without the subjects with polysubstance use and/or a comorbid psychiatric disorder.

	F	p	Partial Eta Squared
BETWEEN SUBJECT EFFECTS			
age	0.332	0.586	0.052
age onset regular use	0.722	0.428	0.107
Dosage change	0.023	0.885	0.004
cumulative dosage at BL. (median split)	1.803	0.228	0.231
gender	7.574	0.033	0.558
WITHIN-SUBJECT EFFECTS			
time point	0.897	0.380	0.130
time point * age	0.030	0.868	0.005
time point * age onset regular use	0.621	0.461	0.094
time point * dosage change	0.005	0.944	0.001
time point * gender	0.058	0.818	0.010
time point * cumulative dosage * gender	0.951	0.367	0.137
hemisphere	1.206	0.314	0.167
hemisphere * age	0.619	0.461	0.094
hemisphere * age onset regular use	0.535	0.492	0.082
hemisphere * dosage change	0.000	0.995	0.000
hemisphere * cumulative dosage	0.126	0.734	0.021

hemisphere * gender	0.361	0.570	0.057
hemisphere * cumulative dosage * gender	0.009	0.928	0.001
time point * hemisphere	0.050	0.830	0.008
time point * hemisphere * age	0.073	0.796	0.012
time point * hemisphere * age onset regular use	0.063	0.811	0.010
time point * hemisphere * dosage change	0.008	0.933	0.001
time point * hemisphere * cumulative dosage	0.039	0.851	0.006
time point * hemisphere * gender	0.144	0.717	0.023
time point * hemisphere * cumulative dosage * gender	0.129	0.732	0.021



Supplementary figure 2. Relationship between age of onset of regular cannabis use and hippocampal volumes ($F(1)=3.72$, $p=.086$, $\eta 2=.29$). On the x-axis the age of onset of regular use is depicted. On the y-axis is the amount of hippocampal volume change (either an increase or a decrease) over time depicted. The closer the data point is to the x-axis, the smaller the amount of hippocampal volume change. The lines depict the regression between change in volume and age of onset (dashed line for the right and solid line for the left hemisphere).



Supplementary Figure 3. Uncorrected results of the development over time for the left and right hemisphere (CB users and HC combined). Dorsal view (top) and ventral view (bottom). This figure is for illustrational purposes, showing the non-significant pattern of change in hippocampal shape. The heat map on the surface indicates the direction of change (follow up minus baseline), with red being growth and green being shrinkage over time. The intensity of the color is an indication of the size of the change, with a larger intensity being associated with a larger change. There is volume increase anteriorly.