Supplement

Longitudinal Changes in Cognitive Functioning and Brain Structure in Professional Boxers and Mixed Martial Artists After They Stop Fighting

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eMethods

1. Structural MRI data processing

T1-weighted image at both visits for each subject were input to the FreeSurfer 6.0 longitudinal processing pipeline[1] to generate subject-specific anatomical labeling from the Desikan-Killiany atlas[2], yielding 68 cortical regions and 12 sub-cortical regions of interest (ROI) for every subject at each visit. The FreeSurfer 6.0 longitudinal processing pipeline significantly reduces the confounding effect of inter-individual morphological variability across multiple time points by using each subject as its own control (https://surfer.nmr.mgh.harvard.edu/fswiki/Longitudinal-Processing). A template volume was first created and ran through FreeSurfer for every subject, and this template further served as the initial guess for the segmentation and surface reconstruction for each data point. Thickness measures of 68 cortical regions and volume measures of 80 cortical and subcortical regions were calculated for each visit. Labels, abbreviations, and corresponding brain lobes of each ROI are listed in the Supplement eTable 1.

In addition, We primarily focused our analyses on cortical thickness measures since the volume measures were highly correlated with head sizes while the cortical thickness measure

were not[3,4]. The approaches that statistically adjust volumes for head sizes further vary among studies, which lack in agreement and lead to inconsistent conclusions[5].

2. Details of linear mixed effect (LME) models

Briefly, in our analysis, due to the overlap/correlation between the scanner variable and the Time variable, an LME model was selected over other simpler models (e.g., linear regressions on differences between time points) to address the scanner covariate. Below is the detailed analysis model:

Cognitive functioning scores (PSS, PSY, VM, RT) and NfL measures:

y ~ 1 + age + education + race + group + Time + group x Time + (1 + Time | Subject)

MRI-derived cortical thickness measures:

y ~ 1 + age + education + race + scanner type + group + Time + group x Time + (1 + Time | Subject)

MRI-derived volume measures:

y ~ 1 + age + education + race + scanner type + TIV + group + Time + group x Time + (1 + Time | Subject)

Abbreviations: PSS: processing speed; PSY: psychomotor speed; VM: verbal memory; RT: reaction time; NfL: neurofilament light; TIV: total intracranial volume;

3. Associations between longitudinal changes in cognitive function and brain structure measures

This follow up longitudinal association analysis was only conducted for structural-MRI derived measures and cognitive function scores with medium effects (Cohen' d (d) $\geq$0.5) in the LME model (i.e., for 4 MRI derived measures (Fig. 3) and 2 cognitive function measures (Fig. 2)). Both cognitive function and brain structure measures were adjusted for covariates in the LME models first ($TP1_{res}$ and $TP2_{res}$). Longitudinal changes (longitudinal loss) were then computed as $\Delta = TP2_{res} - TP1_{res}$. The following linear regression model was used to investigate (1) if any cognitive function changes might be associated with brain structure changes longitudinally in fighters; and (2) if this association (i.e., slope) differed between transitioned and active fighters:

$$\Delta_{\text{score}} \sim 1 + \Delta_{\text{MRImeasure}} + \text{group} + \Delta_{\text{MRImeasure}} \times \text{group},$$

where $\Delta_{\mathrm{MRImeasure}}$ included $\Delta_{\text{right-rMFG-thickness}}$ (d=0.63), $\Delta_{\text{right-rACC-thickness}}$ (d=0.63), $\Delta_{\text{left-mOFC-thickness}}$ (d=0.54), and $\Delta_{\text{right-rMFG-volume}}$ (d=0.66); and Δ_{score} included Δ_{VM} (d=0.79), and Δ_{PSY} (d=0.82).

We were particularly interested in the significance levels (*p*-values) for the term $\Delta_{\mathrm{MRImeasure}}$ (if there might be an association) and the term $\Delta_{\mathrm{MRImeasure}} \times \mathrm{group}$ (if this association differed by group) in the linear regression model. Therefore, the total number of multiple comparisons was 4 ($\Delta_{\mathrm{MRImeasure}}$) x 2 ($\Delta_{\mathrm{score}}$) x 2 (terms) = 16.

As shown in eTable 5, after adjusting for multiple comparisons with the false discovery rate (FDR) method, trend-level associations are observed between Δ_{VM} and $\Delta_{\text{right-rMFG-thickness,}}$ (p_{raw}=0.01, p_{FDR}=0.08). As shown in eFig. 1, this association further differ between transitioned and active fighters (p_{raw}=0.01, p_{FDR}=0.08, eTable 5), with a positive and a negative relationship observed for transitioned and active fighters, respectively. Similarly differential slopes are observed for the association between Δ_{VM} and $\Delta_{\text{right-rMFG-volume}}$ (p_{raw}=0.02, p_{FDR}=0.11, eFig. 1(B), eTable 5).

4. *Apolipoprotein (ApoE)* effect on our results.

In our samples, *ApoE* genotype data are available for 41 transitioned and 41 active fighters as detailed in eTable 6 (A). We repeated our LME analysis with *ApoE* genotype as a covariate (a categorical variable representing detailed *ApoE* genotypes) for these 82 subjects, and eTable 6 (B) list the LME results with (*right*) and without (*left*) *ApoE* covariate. In addition, for all LME analyses, we have plotted raw p-values of the interaction effect before and after including ApoE genotype as a covariate in the LME model in eFig. 2.

As shown in the last column of the eTable 6(B), *ApoE* genotype does not contribute significantly to the LME model expect for the psychomotor scores (p_{raw}=0.03) and right-thalamic volume measure (p_{raw}=0.04). In addition, as shown in eFig. 2, the raw p-values of the interaction effect stay at the same level before and after including *ApoE* as a covariate in the LME model, with a Pearson's correlation value of 0.90. The slight variations of the *p-values* between models might be attributed to the fewer subjects in the LME model with *ApoE.*

eTables

eTable1. Regions of interest (ROIs) in FreeSurfer Segmentation: 68 cortical regions (from Desikan-Killiany atlas) and 12 subcortical regions.

The abbreviations lh and rh represent left and right hemispheres, respectively.

	Left Hemisphere		Right Hemisphere	
	Regions	Lobe	Regions	Lobe
Both cortical thickness and volume measures (N=68)	Left caudalmiddlefrontal	Frontal	Right caudalmiddlefrontal	Frontal
	Left lateralorbitofrontal	Frontal	Right lateralorbitofrontal	Frontal
	Left medialorbitofrontal	Frontal	Right medialorbitofrontal	Frontal
	Left paracentral	Frontal	Right paracentral	Frontal
	Left parsopercularis	Frontal	Right parsopercularis	Frontal
	Left parsorbitalis	Frontal	Right parsorbitalis	Frontal
	Left parstriangularis	Frontal	Right parstriangularis	Frontal
	Left precentral	Frontal	Right precentral	Frontal
	Left rostralmiddlefrontal	Frontal	Right rostralmiddlefrontal	Frontal
	Left superiorfrontal	Frontal	Right superiorfrontal	Frontal
	Left frontalpole	Frontal	Right frontalpole	Frontal
	Left insula	Insula	Right insula	Insula
	Left cuneus	Occipital	Right cuneus	Occipital
	Left lateraloccipital	Occipital	Right lateraloccipital	Occipital
	Left lingual	Occipital	Right lingual	Occipital
	Left pericalcarine	Occipital	Right pericalcarine	Occipital
	Left inferiorparietal	Parietal	Right inferiorparietal	Parietal
	Left postcentral	Parietal	Right postcentral	Parietal
	Left precuneus	Parietal	Right precuneus	Parietal
	Left superiorparietal	Parietal	Right superiorparietal	Parietal
	Left supramarginal	Parietal	Right supramarginal	Parietal
	Left bankssts	Temporal	Right bankssts	Temporal
	Left entorhinal	Temporal	Right entorhinal	Temporal
	Left fusiform	Temporal	Right fusiform	Temporal
	Left inferiortemporal	Temporal	Right inferiortemporal	Temporal
	Left middletemporal	Temporal	Right middletemporal	Temporal
	Left parahippocampal	Temporal	Right parahippocampal	Temporal
	Left superiortemporal	Temporal	Right superiortemporal	Temporal
	Left temporalpole	Temporal	Right temporalpole	Temporal
	Left transversetemporal	Temporal	Right transversetemporal	Temporal
	Left caudalanteriorcingulate	CingulateCortex	Right caudalanteriorcingulate	CingulateCortex
	Left isthmuscingulate	CingulateCortex	Right isthmuscingulate	CingulateCortex
	Left posteriorcingulate	CingulateCortex	Right posteriorcingulate	CingulateCortex
	Left rostralanteriorcingulate	CingulateCortex	Right rostralanteriorcingulate	CingulateCortex
Volume measures (N=12)	Left hippocampus	Limbic	Right hippocampus	Limbic
	Left amygdala	Limbic	Right amygdala	Limbic
	Left thalamus	sub-cortical	Right thalamus	sub-cortical
	Left caudate	sub-cortical	Right caudate	sub-cortical
	Left putamen	sub-cortical	Right putamen	sub-cortical
	Left pallidum	sub-cortical	Right pallidum	sub-cortical

eTable 2. CNS Vital Signs: Linear Mixed Effect results.

P-values from the LME model	Group Effect	Time Effect	Interaction Effect
Processing Speed			2.92E-02
Psychomotor Speed	2.83E-03	1.97E-03	1.32E-04
Verbal Memory	1.74E-04		2.36E-04
Reaction Time			

Significance level (*p*-values) of the group effect, time effect and interaction (group x time) effect from the linear mixed effect (LME) model of each CNS Vital Signs scores. *P*-values<0.05 are listed here.

eTable 3: Neurofilament light (NfL): Linear Mixed Effect results.

***P*-values from the LME model with 45 fighters have NfL at both TP**	Group Effect	Time Effect	Interaction Effect
NfL	2.55E-02		2.09E-02

Significance level (*p*-values) of the group effect, time effect and interaction (group x time) effect from the linear mixed effect (LME) model with 45 fighters (25 transitioned and 20 active fighters) have NfL quantified at both time points. *P*-values<0.05 are listed here.

eTable 4. Structure measures: Linear Mixed Effect results.

P-values from the LME model		Group Effect	Time Effect	Interaction Effect
Thickness	Right Rostral-Anterior-Cingulate		4.54E-03	3.41E-03
	Right Rostral-Middle-Frontal		3.96E-02	2.67E-03
	Left Medial-Orbito-Frontal			1.25E-02
Volume	Right Rostral-Middle-Frontal		6.43E-03	2.16E-03
	Right Thalamus	7.60E-03		2.77E-02

Significance level (*p*-values) of the group effect, time effect and interaction (group x time) effect from the LME model.

eTable 5. Associations between longitudinal changes in cognitive function and brain structure measures

	Δ_{VM}		Δ_{PSY}	
$p_{raw}(p_{FDR})$	$\Delta_{\mathrm{MRI-measure}}$	$\Delta_{\mathrm{MRI-measure}} \times \mathrm{group}$	$\Delta_{\mathrm{MRI-measure}}$	$\Delta_{\mathrm{MRI-measure}} \times \mathrm{group}$
$\Delta_{\text{right-rACC-thickness}}$	0.78 (0.96)	0.71 (0.96)	0.14 (0.32)	0.10 (0.27)
$\Delta_{\text{right-rMFG-thickness}}$	**0.01 (0.08)**	**0.01 (0.08)**	0.75 (0.96)	0.94 (0.97)
$\Delta_{\text{left-mOFC-thickness}}$	0.06 (0.22)	0.28 (0.53)	0.91 (0.97)	0.68 (0.96)
$\Delta_{\text{right-rMFG-volume}}$	0.07 (0.22)	**0.02 (0.11)**	0.30 (0.53)	0.97 (0.97)

Significance levels ($p_{raw}(p_{FDR})$) for the term $\Delta_{\mathrm{MRImeasure}}$ (if there might be an association) and the term $\Delta_{\mathrm{MRImeasure}} \times \mathrm{group}$ (if this association differed by group) in the linear regression model: $\Delta_{\mathrm{score}} \sim 1 + \Delta_{\mathrm{MRImeasure}} + \mathrm{group} + \Delta_{\mathrm{MRImeasure}} \times \mathrm{group}$ are shown here. Raw *p*-values are corrected for 16 multiple comparisons using the false discovery rate (FDR) method.

eTable 6. *ApoE* effect on our results.

(A). ApoE characteristics in 41 transitioned and 41 active fighters.

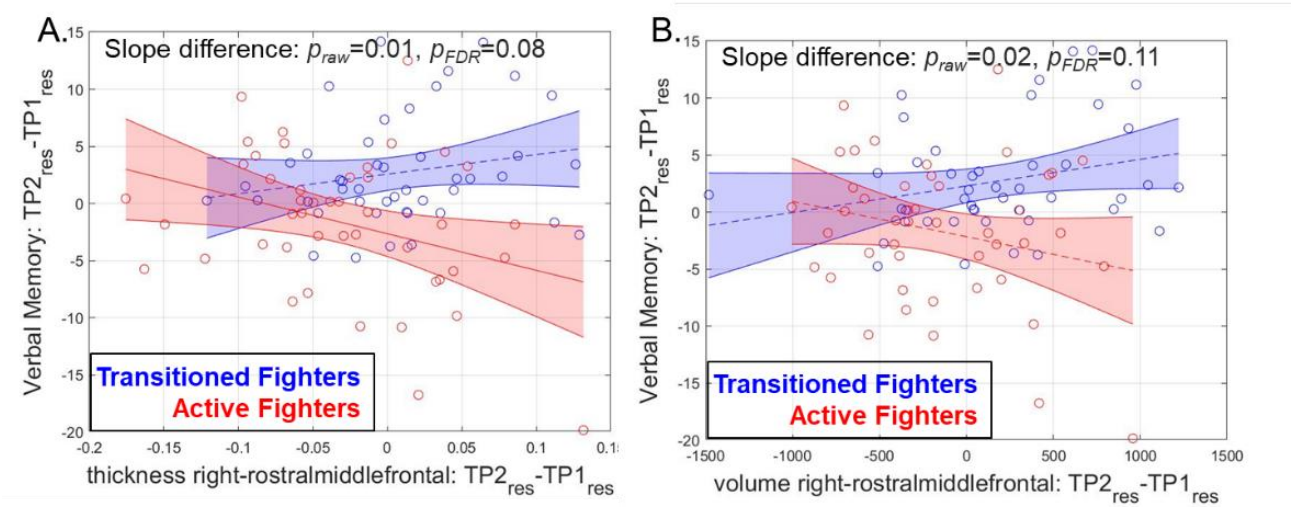
	Transitioned fighters	Active Fighters	Between-group Differences
E2E3	1	6	
E3E3	24	27	
E3E4	13	7	8.78E-02
E4E4	3	1	
Unknown	4	4	

(B). Summarized LME results with *(right)* and without *(left) ApoE* genotype as a covariate.

		Without *ApoE* as a covariate: LME model (#subjects = 90)	With *ApoE* as a covariate: LME model (#subjects = 82)	
		Interaction Effect: p_{raw}	Interaction Effect: p_{raw}	*ApoE* Effect: p_{raw}
Neuro-psychological Scores	Verbal Memory	2.36E-04	9.67E-04	0.48
	Processing Speed	0.03	0.07	0.40
	Psychomotor Speed	1.32E-04	7.91E-04	0.03
Thickness	right-rostralmiddlefrontal	2.67E-03	2.44E-03	0.51
	right-rostralanteriorcingulate	3.41E-03	8.55E-03	0.90
	left-medialorbitofrontal	0.01	3.53E-03	0.90
Volume	right-rostralmiddlefrontal	2.16E-03	3.36E-03	0.04
	right-Thalamus	0.03	0.05	0.10

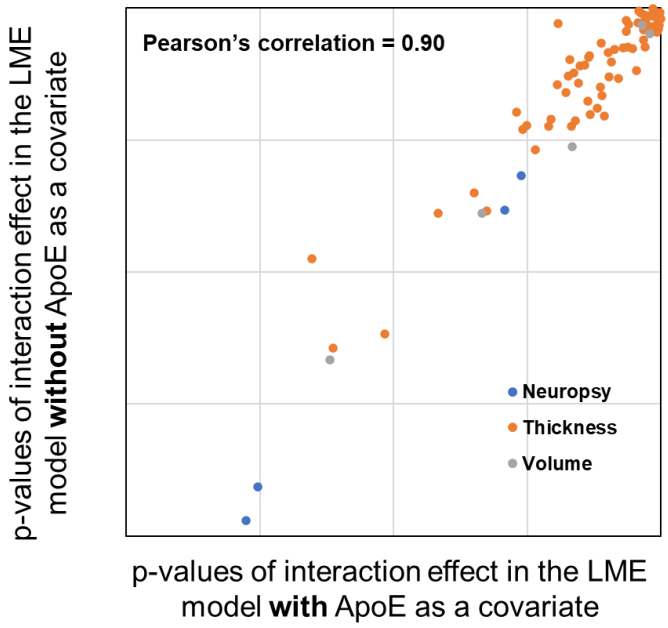
eFigures

eFig. 1. Associations between longitudinal changes in cognitive function and brain structure measures.



eFig.1. Associations between **longitudinal** changes in cognitive function and brain structure measures in transitioned (blue) and active fighters (red). After adjusting for multiple comparisons, trend-level different associations between VM score changes and right rMFG thickness changes were observed for transitioned and active fighters, for both cortical thickness measures (A) and volume measures (B). Only structural-MRI derived measures and cognitive functioning scores with medium effects (Cohen' d≥0.5) in the LME model were followed by this association analysis. *P*-values are listed in the inset boxes.

eFig. 2. Raw p-values of the interaction effect before and after including ApoE genotype as a covariate in the LME model for all neuropsychological (blue), cortical thickness (orange) and volume (grey) measures.



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