

Differential increase of hippocampal subfield volume after socio-affective mental training relates to reductions in diurnal cortisol

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Abstract

The hippocampus is a central modulator of the HPA-axis, impacting the regulation of stress on brain structure, function, and behavior. The current study assessed whether three different types of 3-months mental Training Modules geared towards nurturing a) attention-based mindfulness, b) socio-affective or c) socio-cognitive skills may impact hippocampal organization by reducing stress. We evaluated mental training-induced changes in hippocampal subfield volume and intrinsic functional connectivity, by combining longitudinal structural and resting-state fMRI connectivity analysis in 332 healthy adults. We related these changes to changes in diurnal and chronic cortisol levels. We observed increases in bilateral cornu ammonis volume (CA1-3) following the 3-months compassion-based module targeting socio-affective skills (*Affect* module), as compared to socio-cognitive skills (*Perspective* module) or a waitlist cohort with no training intervention. Structural changes were paralleled by relative increases in functional connectivity of CA1-3 when fostering socio-affective as compared to socio-cognitive skills. Furthermore, training-induced changes in CA1-3 structure and function consistently correlated with reductions in cortisol output. Notably, using a multivariate approach, we found that other subfields that did not show group-level changes also contributed to changes in cortisol levels, suggesting that circuit-level changes within the hippocampal formation are linked to diurnal stress markers. Overall, we provide a link between a socio-emotional behavioural intervention, changes in hippocampal subfield structure and function, and reductions in cortisol in healthy adults.

eLife assessment

This **important** work examines the potential utility of socio-emotional and socio-cognitive mental training on hippocampal subfield structure and function, and cortisol levels. The authors provide **convincing** evidence that CA1-3 volume is sensitive to socio-emotional training, with changes related to function plasticity and cortisol levels. Further, the authors provide evidence of change across all subfields and training modules related to stress.

Introduction

Stress-related disorders rank among the leading causes for disease burden world-wide (1), and the global stress load has increased even more dramatically in recent years (2, 3). It is therefore essential to find ways to efficiently prevent or reduce stress (4). In recent years, research has shown that contemplative mental training programs can be efficient in stress reduction (5–7); for a meta-analysis see (8)), while simultaneously inducing brain plasticity (9–11). It is, however, still unclear which types of mental practices are most efficient in reducing stress and inducing stress-related brain plasticity. Furthermore, stress is a multi-layered construct (12), and most studies focused on stress-related self-reports and questionnaires (8). A less investigated marker in the stress reduction context through contemplative mental training is diurnal cortisol, from which summary indices such as the cortisol awakening response (CAR), the total diurnal output and the diurnal cortisol slope are frequently investigated (13). The steroid hormone and glucocorticoid cortisol is the end-product of the hypothalamic-pituitary-adrenal (HPA) axis and a key player in stress regulation (for reviews see *e.g.*, (14, 15)). Moreover, cortisol is considered an important mediator of the relation between chronic stress and stress-related disease (16, 17). Previous research suggests an association between hippocampal structural integrity and stress related cortisol activity (18, 19), although findings are inconclusive. To close these gaps, we here investigate the differential efficiency of three types of mental training (attention-based, socio-affective and socio-cognitive) on their ability to induce structural as well as functional plasticity of hippocampal subfields and reduce diurnal cortisol levels.

The hippocampus has a high glucocorticoid receptor density (20–23), making this region a target of investigations into stress-related brain changes. Having a three layered allocortex, the hippocampal formation consists of multiple subfields, or zones, starting at the subiculum (SUB) and moving inward to the hippocampus proper; the cornu ammonis (CA1-3), and dentate gyrus (CA4/DG) (24–27). These subfields have unique microstructure (24–28) and participate differently in the hippocampal circuitry (29), likely implicating different functional contributions (30–35). Indeed, intrinsic functional MRI analyses have shown that the hippocampal subfields show functional correlation with a broad range of cortical regions, part of visual, control, and default functional networks (28, 35–38). Hippocampal subfield volumes and associated intrinsic functional connectivity have been shown to be heritable (36, 39), indicating that individual variation in subfield structure and function is, in part, under genetic control. Other lines of research have reported hippocampal structure and function to be highly sensitive to contextual factors, such as stress (23). Mediated through its dense network of glucocorticoid receptors, the hippocampus transmits the negative feedback signals of a wide range of glucocorticoid levels on HPA axis activity (22). Through this inhibitory role on HPA axis dynamics, it is linked to emotional reactivity (40), stress sensitivity (19, 41–43), and causally involved in a variety of stress-related disorders (44).

Previous brain imaging research has examined the relationship between cortisol activity and hippocampal structure and function. Most of this research measured saliva cortisol levels to gauge the diurnal cortisol profile. Thus, a reduced cortisol awakening response, the response to the anticipated demands of the upcoming day (45), has been associated with smaller hippocampal volume in healthy individuals (46–48) and different psychiatric (49, 50) and metabolic (48, 51) conditions. In fact, the examination of patients with temporal lobe damage suggested that hippocampal integrity may be a necessary condition for the proper mounting of the CAR (52, 53). Next, to changes in hippocampal structure, alterations in hippocampal functional connectivity have been reported to be associated with changes in cortisol levels (54, 55). There is also contrary work showing associations between elevated awakening, evening, diurnal, or 24-hour cortisol levels in healthy elderly with age-related hippocampal atrophy (56–59) and, again, samples with psychiatric conditions (60, 61). While such inconsistencies in previous neuroimaging work may reflect the fact that different indices of diurnal cortisol tap different facets of HPA axis regulation, the samples studied have been diverse in terms of health status, small in size, and largely cross-sectional. In addition, associations between stress and hippocampal structure and function over time are incompletely understood. Thus, longitudinal studies, such as mental training studies aimed at stress reduction that repeatedly assess both brain and cortisol release, may help to better understand the dynamic relationships between stress, cortisol, and hippocampal structure and function.

In recent years, contemplative mental training interventions, such as the mindfulness-based stress reduction (MBSR) program (62) or compassion-focused therapy (63), have gained in popularity as potential therapeutic tools to improve mental and physical health (64) and reducing stress (8). These mental training interventions can have a positive impact on the practitioner's stress sensitivity, making them a suitable model to investigate the interrelationship between training-related changes in hippocampal structure, function, and cortisol output. Next to reductions in reactive measures following acute psychosocial stress induction in the laboratory (7), reduced subjective-psychological stress load is the most widely reported outcome (for a review, see (8)). Evidence for lower diurnal cortisol output stems mainly from mindfulness-based interventions, notably MBSR, for which reductions in CAR and afternoon/evening cortisol levels have been reported in healthy and diseased individuals (65–67). Moreover, other work in the current sample has shown that hair cortisol and cortisone are reduced through mental practice (5). Hair cortisol measurements have been suggested to provide a window into long-term impact of cortisol exposure (68). These findings are contrasted by numerous null results (for meta-analyses see: (69, 70)), possibly due to modest samples sizes and mixed effects of different training contents on stress-related processes. Furthermore, 8-weeks mindfulness programs such as MBSR and others typically cultivate different types of mental practices, making it difficult to understand which type of mental practice is most efficient in reducing different types of outcomes, including various stress-markers (see also (5–7)).

The current study, therefore, investigated differential effects of distinct mental training practices onto the association between changes in hippocampal subfields and underlying stress-related diurnal cortisol profiles changes in the context of a large-scale 9-month mental training study, the *ReSource Project* (71). We explored impact of long-term exposure to stress onto hippocampal subfields as a function of mental training in a subset of individuals (5). Healthy participants attended three 3-months Training Modules termed *Presence* (cultivating attention and interoceptive awareness), *Affect* (cultivating compassion, prosocial motivation and dealing with difficult emotions) and *Perspective* (cultivating metacognition and perspective-taking on self and others) (**Figure 1**). *Presence* resembles typical mindfulness-based interventions, but excludes socio-emotional or socio-cognitive practices (62, 72). By contrast, *Affect* and *Perspective* target social skills through the training of either socio-emotional and motivational skills such as empathy, compassion and care (*Affect*) or socio-cognitive skills such as perspective taking on self and others (*Perspective*). In previous work, stemming from the same participant sample as examined here, we found a reduction in CAR specifically after the training of socio-affective capacities (6), and of

acute stress reactivity after the training of socio-affective or socio-cognitive capacities (7 [↗](#)). Alternatively, but also in the current sample, different types of mental practices equally reduced hair cortisol levels, a marker of long-term stress (5 [↗](#)). This suggests that the content of mental training has a specific effect on daily cortisol changes but not on long-term stress levels. Our group could also show differentiable training-related changes in cortical structure and intrinsic functional organization following the three *ReSource* project Training Modules, illustrating the existence of training-related structural plasticity of the social brain (9 [↗](#), 73 [↗](#)). Domain-specific changes in hippocampal subfield structure and intrinsic functional connectivity, and how these relate to mental training specific changes in stress-related diurnal cortisol output, have not yet been studied. We, therefore, examined whether module-specific changes in diurnal cortisol levels may relate to specific structural and intrinsic functional changes in different hippocampal subfields and functional resting state data.

We evaluated the longitudinal relationship between hippocampal subfield volumetry, a quantitative index of hippocampal grey matter, and studied whether volumetric changes were paralleled by subfields' resting-state functional connectivity in a large sample of healthy adults participating in the *ReSource Project* (71 [↗](#)). This enabled us to evaluate training effects on hippocampal structure, function, and their associations with cortisol as a function of mental training targeting either attention-based mindfulness (*Presence*), socio-affective (*Affect*), or socio-cognitive (*Perspective*) skills. Hippocampal structure was quantified via a surface-based multi-template algorithm that has been shown to perform with excellent accuracy in healthy and diseased populations of a comparable age range as the currently evaluated cohort (74 [↗](#)). Such a model is good to represent different subfields *in vivo*, which have a differentiable structure and function (75 [↗](#), 76 [↗](#)), and thus may show differentiable changes as a function of mental training. We expect that assessment of hippocampal sub-regions may help to accurately map circuit plasticity as a result of potential stress reduction, and to observe that changes in hippocampal structure are paralleled by changes in functional connectivity of hippocampal subfield functional networks. To model the interplay between individual-level correspondence in hippocampal and stress markers, we assessed the association of changes in hippocampal structure and function with changes in several indices of diurnal cortisol release.

Results

We analyzed structural MRI, resting-state functional MRI, as well as cortisol-based stress markers from the large-scale *ReSource Project* (71 [↗](#)). For details, see <http://resource-project.org> [↗](#) and the preregistered trial <https://clinicaltrials.gov/ct2/show/NCT01833104> [↗](#).

In the *Resource* study, participants were randomly assigned to two training cohorts (TC1, N=80; TC2, N=81) and underwent a 9-months training consisting of three sequential Training Modules (*Presence*, *Affect*, and *Perspective*) with weekly group sessions and daily exercises, completed via cell-phone and internet platforms (**Figure 1** [↗](#), **Table 6** [↗](#)-**8** [↗](#), *Materials and Methods and Supplementary Materials for more details*). TC1 and TC2 started their training regimen with the *Presence* module, and then underwent the latter two modules in different orders (TC1: *Affect-Perspective*; TC2 *Perspective-Affect*) to serve as active control groups for each other (**Figure 1C** [↗](#)). Another active control group (TC3; N=81) completed three months of *Affect* training only. Additionally, a matched test-retest control cohort did not undergo any training (RCC, N=90). All participants were examined at the end of each 3-months module (T₁, T₂, T₃) using 3T MRI, behavioral and peripheral-physiological measures that were identical to the baseline (T₀) measures.

Change in bilateral CA1-3 volume following Affect mental training

The above design allowed us to examine whether the volume of hippocampal subfields shows increases or decreases following the distinct Training Modules. We tracked longitudinal changes in hippocampal subfield volumes using mixed-effects models (74). Excluding participants with missing or low quality structural and functional data, the sample included 86 individuals for *Presence*, 92 individuals for *Affect*, 83 individuals for *Perspective*, and 61 *active controls* (*Affect*) with hippocampal change scores. We included 164 change scores of *retest controls* over T_1 , T_2 , T_3 . To study whether there was any training module-specific change in hippocampal subfield volumes following mental training, we compared training effects between all three Training Modules (*Presence*, *Affect*, and *Perspective*). Main contrasts were: *Presence* vs *Active control* (between subjects) and *Affect* vs *Perspective* (within subjects). Supplementary comparisons were made vs retest controls and within training groups. We observed relative increases in right cornu ammonis 1-3 (CA1-3), but not in subiculum (SUB) or CA4 and dentate gyrus (CA4/DG) subfields, following *Affect* versus *Perspective* training (left: $t=2.360$, $p=0.019$, $FDR(q)>0.1$, Cohens $D=0.282$; right: $t=2.930$, $p=0.004$, $q=0.022$, Cohens $D=0.350$), that could be attributed to subtle increases ($p<0.05$) in bilateral CA1-3 volume following *Affect* (left: $t=2.495$, $p=0.013$, $q=0.08$, $M: 25.511$, $std: 130.470$, $CI [-1.509\ 52.531]$; right: $t=2.374$, $p=0.018$, $q>0.1$, $M: 40.120$, $std: 181.300$, $CI [2.573\ 77.666]$), and subtle decreases ($p<0.05$) in right CA1-3 volume following *Perspective* (left: $t=-1.143$, $p>0.1$, $q>0.1$, $M:-23.048$, $std: 137.810$, $CI [-53.139\ 7.043]$; right: $t=-2.118$, $p=0.035$, $q>0.1$, $M:-39.602$, $std: 208.470$, $CI [-85.122\ 5.917]$). We did not observe differences between *Presence* and the *Active control* cohort, *Affect* TC3. Overall, for all hippocampal subfields, findings associated with volume increases in CA1-3 following the *Affect* training were most consistent across timepoints and contrasts (**Supplementary File 1a-g**). Moreover, associations between CA1-3 and *Affect*, relative to *Perspective*, seemed to go largely above and beyond changes in the other subfields (left: t -value: 2.298 , $p=0.022$, $Q>0.1$; right: t -value: 3.045 , $p=0.0025$, $Q=0.015$, see further **Supplementary File h**). We observed no overall change in hippocampal subfield volume following mental training of nine months (**Supplementary File 1i**). Although stereotaxic normalization to MNI space would in theory account for global sex differences in intra-cranial volume (ICV), we still observed sex differences in various subfield volumes at baseline. Yet, accounting for ICV did not impact our main results, suggesting changes in CA1-3 following *Affect* were robust to sex differences in overall brain volume (**Supplementary File 1j**).

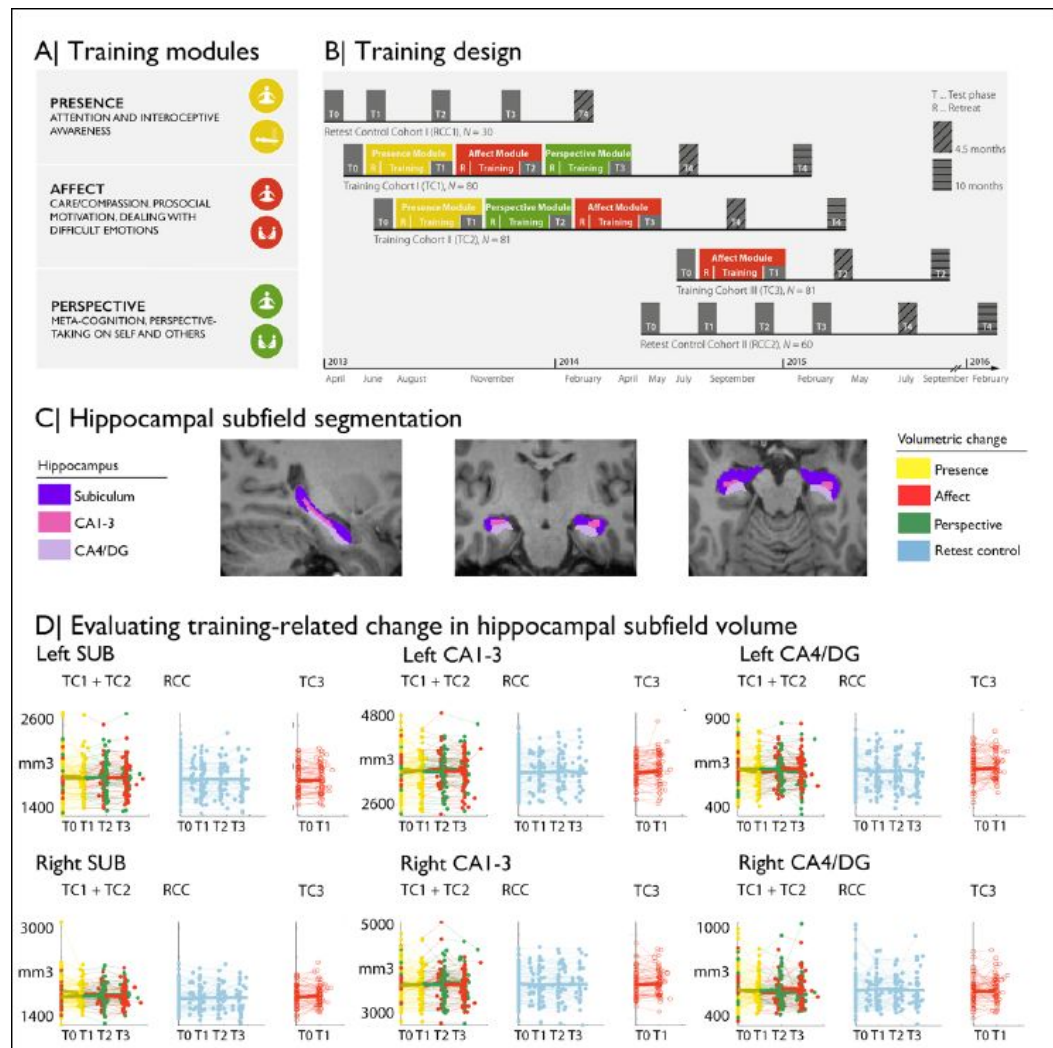


Fig. 1

Training induced plasticity of hippocampal subfield volume.

A) Training modules; B) Training design; C) Subfield volumes in left and right hemispheres across individuals and timepoints; D) Scatterplot of subfield volumes as a function of timepoints and training cohorts.

Increased functional connectivity of CA1-3 following socio-affective versus socio-cognitive mental training

Subsequently, we studied whether volumetric change in hippocampal CA1-3 would show corresponding changes in intrinsic function following the *Affect* mental training. To probe the CA1-3 functional connectivity networks per subfield, we mapped the top 10% of normalized functional connections at baseline. Functional connectivity was strongest to medial prefrontal regions, precuneus extending to posterior cingulate, anterior temporal regions and angular gyrus (CA1-3: **Figure 2**; see **Supplementary Materials** for other subfields). Evaluating functional connectivity changes, we found that the right CA1-3 functional network showed differential changes when comparing *Affect* training to *Perspective* training (2.420, $p=0.016$, $q=0.032$, Cohens $D=0.289$), which could be attributed to subtle ($p<.05$) decreases in right CA1-3 mean FC following *Perspective*

($t=-2.012$, $p=0.045$, $q>0.1$, $M:-0.024$, $std: 0.081$, $CI [-0.041 -0.006]$), but not *Affect* training ($t=1.691$, $p=0.092$, $q>0.1$, $M: 0.010$, $std: 0.098$, $CI [-0.01 0.031]$); changes were not present when comparing *Affect* training versus retest control (Table 1 and Supplementary File 1k-q). Comparing *Affect* TC3 relative to *Presence* training, we did not observe changes (Table 1). No other subfield showed differential changes in main contrasts within its functional network.

<i>Affect</i> TC3 vs <i>Presence</i>	LCA1-3	RCA1-3
<i>t-value</i>	0.366	-0.411
<i>p- and q-value</i>	$p>0.1$, $q>0.1$	$p>0.1$, $q>0.1$
<i>Cohens D</i>	0.052	-0.058
<i>Affect</i> vs <i>Perspective</i>		
<i>t-value</i>	0.137	2.420
<i>p- and q-value</i>	$p=0.891$, $q>0.1$	$p=0.016$, $q=0.032$
<i>Cohens D</i>	0.016	0.289

Table 1.

Changes in mean CA1-3 functional network between training and active control cohorts [T0-T1] and [T1-T3].

Exploring whether particular regions within the CA1-3 network showed alterations in intrinsic functional connectivity when comparing *Affect* to *Perspective*, we investigated connectivity changes within regions of the subfields' functional networks. Left CA1-3 connectivity showed decreases in connectivity to left posterior insula when comparing *Affect* to *Perspective* training ($FDRq<0.05$; $t=-3.097$, $p=0.003$, $Cohens D=-0.370$). On the other hand, we observed connectivity increases between right CA1-3 to right mPFC for the same contrast ($FDRq<0.05$; $t=3.262$, $p=0.002$, $Cohens D=0.389$). No other subfield's functional connectivity showed alterations when comparing *Affect* to *Perspective* or *Presence* to *Affect* TC3. These analyses indicate an overlap between volumetric increases and functional alterations when comparing changes following socio-affective mental training in CA1-3. In particular, the moderately consistent CA1-3 volume increases following *Affect* training were complemented with differential functional connectivity alterations of this subfield when comparing *Affect* to *Perspective* training.

Fig. 2

Training induced plasticity of CA1-3 functional connectivity.

A) *upper*: CA1-3 functional connectivity at baseline, top 10% of regions representing the CA1-3 functional network; *lower*: scatter plot visualizing change within the CA1-3 network across timepoints and groups; networks and scatters of SUB and CA4/DG are available in the supplements; B) Regional change within CA1-3 functional network *Affect* versus *Perspective* ($FDRq < 0.05$); *right*: scatter plot visualizing mean change within the CA1-3, $FDRq < 0.05$ regions across timepoints and groups.

Association between change in subfield volume, function, and stress markers

Last, we probed whether group-level changes in hippocampal subfield CA1-3 volume would correlate with individual-level changes in diurnal cortisol indices (*Presence*: $n = 86$; *Affect*: $n = 92$; *Perspective*: $n = 81$), given that the hippocampal formation is a nexus of the HPA-axis (19). We took a two-step approach. First, we studied univariate associations between cortisol and subfield change, particularly focusing on the *Affect* module and CA1-3 volume based on increases in CA1-3 volume identified in our group-level analysis. We observed that increases in bilateral CA1-3 following *Affect* showed a negative association with change in total diurnal cortisol output (operationalized as the area under the curve with respect to ground; AUC_g) (left: $t = -2.237$, $p = 0.028$, $q = 0.056$; right: $t = -2.283$, $p = 0.025$, $q = 0.05$), indicating that with a reduction in stress-levels as measured by AUC_g , there were increases in CA1-3 volume. *Post-hoc* analyses indicated no other subfield showed an association with AUC_g , or with any of the other cortisol indices, below $p < 0.05$ (Supplementary File 1r). Assessing the associations between cortisol indices and the right CA1-3 subfield functional networks in *Affect* ($n = 92$), we could not observe individual level modulation of diurnal cortisol markers and group-level effects (right CA1-3 functional network change and cortisol markers or within the PI or mPFC ROI, Table 3 and Supplementary File 1s). Yet, we observed positive associations between mean functional network of *left* CA1-3 and diurnal slope ($t = 2.653$, $p = 0.01$, $q = 0.02$) and AUC_g ($t = 2.261$, $p = 0.026$, $q = 0.052$), Supplementary File 1t. When assessing whether particular regions within the CA1-3 network showed alterations in intrinsic functional connectivity, we observed that AUC_g modulated increases in connectivity between left CA1-3 and parietal occipital area ($q < 0.05$). These analyses extend group-level observations regarding the relation between socio-affective mental training and CA1-3 structure to the individual-level. Again, we observed some consistency in structure and function in case of CA1-3. We did not observe alterations in CA1-3 volume in relation to change in cortisol markers in *Presence* or *Perspective*. Yet, for *Presence* we observed association between slope and LCA4/DG change ($t = -2.89$, $p = 0.005$, $q = 0.03$), (Supplementary Tables File 1uv). In case of intrinsic function, we also did not observe alterations in CA1-3 in relation to change in cortisol markers in *Presence* or *Perspective*, nor in other subfields (Supplementary File 1wx). When evaluating overall associations between diurnal cortisol change in CA1-3 volume in all modules combined, (*Presence*, *Affect*, and *Perspective*), we observed comparable patterns as for *Affect* only, further underscoring the association between cortisol markers and CA1-3 (Figure 3B; Supplementary File 1y and z). Last, we explored whether associations of subfield volume were found with levels of hair cortisol, a long-term marker of systemic cortisol exposure, in a sub-sample of $N = 44$ participants repeatedly tested across modules (*Presence*, *Affect*, and *Perspective*), based on previous observations of domain-general effects of mental training on cortisol and cortisone (5). We identified consistent associations between increases in LCA1-3 volume and intrinsic function and hair cortisol decreases (volume: $t = -2.574$, $p = 0.011$, $q = 0.022$, function: $t = -2.700$, $p = 0.008$, $q = 0.016$). Exploring

effects in subfields other than CA1-3 we revealed associations between right CA4/DG volume and cortisol ($t=-3.138$, $p=0.002$, $q = 0.01$) and left SUB function ($t=-2.890$, $p=0.005$, $q = 0.03$) (**Figure 3B**; **Supplementary File 1za and zb**).

	LCA1-3	RCA1-3
CAR	-0,355, $p>0.1$	-1,543, $p>0.1$
Slope	-0,878, $p>0.1$	-1,245, $p>0.1$
AUC _g	-2,237, $p=0.028$, $q=0.056$	-2,283, $p=0.025$, $q=0.05$

Table 2.

Correlating change in CA1-3 subfield volume and diurnal cortisol indices in *Affect*.

	LCA1-3	RCA1-3
CAR	-0,476, $p>0.1$	-0,425, $p>0.1$
Slope	2,653, $p=0.009$, $q=0.018$	0,773, $p>0.1$
AUC _g	2,261, $p=0.026$, $q=0.052$	0,024, $p>0.1$

Table 3.

Correlating change in CA1-3 subfield functional network and diurnal cortisol indices in *Affect*.

Fig. 3.

Associations between changes in structure and function of hippocampal subfield volume and markers of stress change.

A). *Upper left*: Correlation between hippocampal subfield volume change in *Affect* and CAR, slope, and AUC markers of stress change, *Upper right*: Correlation between hippocampal subfield intrinsic functional change in *Affect* and CAR, slope, and AUC markers of stress change, *middle*: Scatter plots visualize the correlation between volume change and cortisol marker change (below $p<0.05$), *bottom*: region level change within left CA1-3, $FDRq<0.05$. CA1-3 is the focus of this analysis based on our group-level findings and highlighted with boxes in A; B). *Upper*: Overall impact of diurnal cortisol markers on hippocampal subfield volume and function over *Presence*, *Affect* and *Perspective*; *Lower*: Overall impact of hair cortisol markers on hippocampal subfield volume and function over *Presence*, *Affect* and *Perspective*

Second, to further explore the question whether there is concordant cortisol-related structural and functional plasticity in the hippocampus we adopted a multivariate partial least square approach, with 1000 permutations to account for stability (77, 78) and bootstrapping (100 times) with replacement. We ran two complementary models including all Training Modules whilst regressing out age, sex and random effects of subject. First, we focused on the bilateral CA1-3, as this region showed increases in volume following *Affect* training and differential change between *Affect* and *Perspective* training in our resting state analyses. In the second model included structural and functional data of all subfields. Both models included all stress markers. We found that both models could identify significant associations between cortisol stress markers and hippocampal plasticity ($FDRq<0.05$), and that in particular *Affect* showed strongest associations with the latent markers for CA1-3 (Table 5). Both analyses showed inverse effects of subfield structure and function in relation to stress markers and both slope and AUC changes showed strongest associations with the latent factor.

	LC1	Overall	Presence	Affect	Perspective
CA1-3	$p<0.01$, 67%	$r=0.20$	$r=0.17$	$r=0.27$	$r=0.16$
all	$p<0.01$, 71%	$r=0.24$	$r=0.16$	$r=0.30$	$r=0.26$

Table 5.

Multivariate PLS analyses linking cortisol markers to hippocampal subfield volume and function.

Fig. 4.

Multivariate associations between changes in structure and function of hippocampal subfield volume and markers of stress change in Affect.

A). Multivariate associations between bilateral CA1-3 volume and intrinsic function and stress markers. *Left*: Scatter of loadings, colored by Training Module; *Right upper*: individual correlations of stress markers; *Right lower*: individual correlation of subfields; B). Multivariate associations between all subfields' volume and intrinsic function and stress markers. *Left*: Scatter of loadings, colored by Training Module; *Right upper*: individual correlations of stress markers; *Right lower*: individual correlation of subfields.

Discussion

The goal of the current work was to investigate the effects of different types of mental training regimens on stress-related changes in the human hippocampus. The hippocampal formation is a highly plastic allocortex implicated in stress and emotional reactivity (14, 15, 19). In this study, we used automated segmentation to examine if the volumes of hippocampal subfields (SUB, CA1-3, CA4/DG) change in a large healthy sample over a 9-month longitudinal mental training study, the *ReSource* project (71). We investigated whether three different interoceptive and social mental Training Modules could lead to changes in hippocampal subfield volume. Additionally, we explored if these changes were associated with alterations in both intrinsic brain function and stress-related physiological changes, as indicated by shifts in diurnal cortisol levels due to training.

When comparing the differential efficiency of the 3-months mental Training Modules *Presence*, *Affect*, and *Perspective* against each other and a retest-control group on hippocampal subfield structure, we observed consistent increases in bilateral CA1-3 volume following socio-emotional *Affect* training relative to socio-cognitive *Perspective* training and no training in retest controls. Moreover, alterations in structure were mirrored by changes in functional connectivity of right CA1-3 following *Affect* versus *Perspective* training. In particular, we observed relative increases of functional connectivity between right CA1-3 and mPFC, and decreases between left CA1-3 and posterior insula, mainly driven by changes in connectivity following *Perspective* training. Evaluating training-related changes in diurnal cortisol output (cortisol awakening response, total diurnal output and diurnal slope), we observed that bilateral CA1-3 volume increases correlated with decreases in total diurnal cortisol output (assessed as the area under the curve with respect to ground, AUC_g, sampled on 10 occasions over two consecutive days). Intrinsic connectivity of CA1-3 following *Affect* showed a positive association with left CA1-3 network change and diurnal slope and total diurnal cortisol output, where the latter associated with increased connectivity between left CA1-3 and parietal-occipital area. Interestingly, these associations were similar when combining Training Modules, suggesting the association between CA1-3 and diurnal cortisol markers is present irrespective of training content. Moreover, we additionally observed consistent associations between left CA1-3 and hair cortisol, a chronic stress marker, across trainings in a sub-sample of the current study. Last, performing multivariate analysis we again observed associations between CA1-3 volume and function plasticity and stress change, strongest in *Affect*. Yet combining all subfields in a single model indicated that other subfields also link to stress alterations, indicating that ultimately circuit-level alterations within the hippocampal formation relate to latent changes in diurnal stress markers across Training Modules.

Our longitudinal, multi-modal approach could thus show that compassion-based mental training alters CA1-3 structure. Second, training-based increases in CA1-3 volume related to decreases in total diurnal cortisol release, suggesting that mental training and CA1-3 volume changes are linked

to cortisol release. The results regarding changes in functional connectivity profiles were not as straightforward, but they did reveal a distinction between *Affect* and *Perspective* mental training in the CA1-3 region. Moreover, CA1-3 intrinsic functional change was associated with changes in diurnal cortisol slope and release, and long-term cortisol exposure. While the experimental nature of our training study allows concluding that CA1-3 structure changed as a function of *Affect* training, and that individual differences in CA1-3 structural change corresponded to cortisol release change, we cannot make any claims about which training-induced change caused the other. Thus, it is possible that, owing to the *Affect* module, the activation of emotion/motivation-related functional processes is key to reducing the daily stress load and associated cortisol release (79, 80). Such reduction in cortisol levels may then explain the observed downstream brain alterations. According to this interpretation, changes in CA1-3 volume may come secondary to stress reduction and consequently alterations in cortisol release following compassion training. Alternatively, emotion/compassion training may specifically targets CA volume and function, and, as per its role as the central break of the HPA axis, improves its capacity to inhibit cortisol release. This explanation could explain the lack of *average* diurnal cortisol (i.e., AUC_g) change following *Affect* training *per se* (6), as it may be relevant for individual variations in brain change and thus be more difficult to detect based only on average change per module. In sum, it is likely that observed alterations in hippocampal structure and function, as well as their associations with diurnal cortisol change, are not explained by a single mechanism, but rather result as a combination of different factors. This interpretation is also supported by our multivariate observations. For example, given the anatomical and functional complexity of the hippocampal formation (26, 33) as well as the multifaceted cognitive processes underlying stress, it seems plausible that our observations are an emergent effect of multiple, cognitively distinct, functional sub-processes. Thus, future studies may directly test the potential specificity of the interrelationship between stress on the hippocampus using further targeted measurements.

The observed increases in CA1-3 volumes following socio-affective mental training were small-sized effects. However, findings were consistent when independently assessing the left and right hippocampus subfields. In particular, we observed that increases in CA1-3 volume after *Affect* training corresponded to a decrease in total diurnal cortisol as well as hair cortisol output. These results can be interpreted in line with the mainly inhibitory role of the hippocampus in stress regulation (20–22, 81). Specifically, the hippocampus is involved in the negative feedback inhibition of the HPA axis. Mineral- and glucocorticoid receptors are present in abundance in hippocampal neurons, from where they transmit the negative feedback signals of a wide range of glucocorticoid levels on HPA axis activity (22). The extremely high numbers of mineral- and glucocorticoid receptors make the hippocampus a prominent target for the neuro-toxic effects of glucocorticoids (82–84). In particular the CA1 may be susceptible to stress-based environmental effects due to synaptogenesis associated with NR2B subunits of glutamate receptors (NMDAR)(85). Along these lines, sustained exposure to high glucocorticoid levels was shown to relate to calcium influx, and may produce CA3 pyramidal neuronal damage, which has been reported in rodents and tree shrews (86–88). Next to demonstrating a consistent relationship between total daily cortisol output and hippocampal structure, the absence of univariate findings for cortisol awakening response (CAR), diurnal slope or hair cortisone levels may a divergence in the sensitivity of alternative cortisol-based stress markers to structural neuroimaging markers. It is of note that the current work relies on a segmentation approach of hippocampal subfields including projection to MNI template space, an implicit correction for total brain volume through the use of a stereotaxic reference frame. Some caution for this method may be warranted, as complex hippocampal anatomy can in some cases lead to over- as well as underestimation of subfield volumes, as well as subfield boundaries may not always be clearly demarcated (89). Future work, studying the hippocampal surface at higher granularity, for example through unfolding the hippocampal sheet (35, 76, 90, 91), may further help with both alignment and identification of not only subfield-specific change but also alterations as a function of the hippocampal long axis, a key dimension of hippocampal structural and functional variation that was not assessed in the current work (33, 92).

Structural MRI findings were complemented by the separate assessment of task-free (“resting-state”) functional connectivity networks. Identification of networks that show coupled spontaneous brain activity through resting-state fMRI is currently considered an effective approach to study whole-brain functional connectivity (93–95). In the current cohort, we could demonstrate widespread patterns of hippocampal functional connectivity to mesiotemporal, lateral temporal, together with anterior as well as posterior midline regions, lateral temporo-parietal, and dorsolateral prefrontal cortices - a pattern in excellent accordance to previous studies probing hippocampal functional connectivity at rest in healthy populations (28, 35, 38, 96, 97), and outlining “mesiotemporal” components of default-mode networks (98, 99). Assessing modulations of connectivity by mental training, we could provide independent, yet weak, support for a specific relationship of the socio-affective *Affect* training, relative to socio-cognitive *Perspective* Training Modules, on hippocampal network embedding. In particular, we observed an increased functional integration of the right CA1-3 with medial prefrontal cortical regions (mPFC) in individuals following *Affect* relative to *Perspective* training. Studies in rats and non-human primates have demonstrated a high density of glucocorticoid receptors in the mPFC (100, 101). Accordingly, the mPFC, like the hippocampus, was shown to play a key role in HPA-axis regulation (50, 81, 102, 103). In a previous positron emission tomography study, glucose metabolism in the mPFC was negatively associated with acute stress-induced salivary cortisol increases; notably, the authors observed a negative metabolic coupling between mPFC areas and the mesiotemporal lobe (104). In related work on isocortical changes in structure and intrinsic function following the *ReSource* training, we have observed structural changes in insular, opercular and orbitofrontal regions following *Affect* training (9, 73). At the same time, we observed little change in large-scale functional organization, relative to changes observed following *Presence* and *Perspective* training. Previous work has implicated the hippocampal formation at the nexus of multiple large-scale networks and cortical organization (28, 92). Indeed, it may be that particular changes in the CA1-3 are central in coordinating the signal flow within the hippocampal complex, coordinating the balance between large-scale association networks in the isocortex (28). Integrating this with our empirical observation of *Affect* training taking up a regulatory or stabilizing functional role, relative to *Perspective* and *Presence* training, it is possible that such alterations are orchestrated by adaptive processes (105). Future work may be able to further disentangle the causal relationship between iso- and allo-cortical structure and function, and the role of specific hippocampal subfields.

Using univariate approaches, we could observe that training-induced HC volume increases following socio-affective mental training overlapped with reductions in cumulative diurnal cortisol release. Additionally, we observed functional connectivity decrease between left CA1-3 and parietal-occipital area in individuals showing reduced diurnal cortisol release and overall connectivity decreases of left CA1-3 relating to reductions in diurnal cortisol slope. Importantly, these associations could be found also when including *Presence* and *Perspective* in our analysis, suggesting of a domain-general relationship between diurnal cortisol alterations and CA1-3 volume and function. In line with our observations in univariate analysis, we found multivariate associations between hippocampal subfield volume, intrinsic function and cortisol markers. Again, the contribution of volume and intrinsic function was inverse. This may possibly relate to the averaging procedure of the functional networks. Combined, outcomes of our univariate and multivariate analyses point to an association between change in hippocampal subfields and stress markers, and that these changes, at the level of the individual, ultimately reflect complex interactions within and across hippocampal subfields and may capture different aspects of diurnal stress. Future work may more comprehensively study the plasticity of the hippocampal structure, and link this to intrinsic functional change and cortisol to gain full insights in the specificity and system-level interplay across subfields, for example using more detailed hippocampal models (90). Incorporating further multivariate, computational, models is needed to further unpack and investigate the complex and nuanced association between hippocampal structure and function, in particular in relation to subfield plasticity and short and long-term stress markers. In line with our multivariate observations, in other work from the *ReSource* study we observed

mixed specificity of stress-reducing effects as a function of mental training. For example, both social modules, that is the *Affect* and *Perspective* trainings, reduced acute cortisol reactivity to a psychosocial stressor (106), which is considered a dynamic state of HPA axis activity (7). Regarding the CAR, only *Affect* training was able to reduce this dynamic cortisol response to awakening, known to reflect anticipatory stress (6). Lastly, regarding hair cortisol, a long-term measure of systemic stress, all Training Modules were shown to be equally effective in stress reduction over a training period of three to nine months (5). In our work we observed a consistent association between left CA1-3 volume and functional increases and hair cortisol decreases, hinting at a potential relationship between CA1-3 and both short-term and long-term stress level changes.

Overall, different types of mental training result in stress reduction (e.g. (5–7)). In a recent paper we argue that the variable pattern of mental training effects on different cortisol indices may be explained by the functional roles of these indices (6). Thus, indices reflecting dynamic HPA axis properties, such as acute stress reactivity and the CAR, were suggested to change with *Affect* and *Perspective* training (also see (7)). Hair cortisol as a marker of cumulative stress load likely reflecting the low-grade and continuous strain inherent to daily hassles (107–109), was contrarily suggested to change independent of training type (also see (5)). The current findings do not necessarily contradict this reasoning, due to differences in interpretation of group-level and individual-level changes. Although we observed that CA1-3 volume was selectively increased by socio-affective mental training at the group level, and that individual differences in CA1-3 volume increase within the *Affect* module correlated with reduced diurnal cortisol release, the pattern linking bilateral CA1-3 volume increases with reduced diurnal cortisol release was also present when all modules were combined. Similarly, in follow-up analysis on functional alterations of hippocampal subfields, we could observe group-level increases in connectivity to mPFC for right, but not left, CA1-3, when comparing socio-affective and socio-cognitive training. Though right CA1-3 group-level changes did not link to individual level change in cortisol markers following *Affect* training, individual level changes in left CA1-3 corresponded to changes in cortisol markers, again following *Affect* but also across all practices combined. Thus, we cannot at this point derive a consistent pattern of how mental training influences different indices of cortisol activity, yet we do find a consistent change in CA1-3 following *Affect* training, and observe domain-general patterns of change associations between CA1-3 and cortisol markers, indicating CA1-3 may play a central role within the context of *Affect* training and diurnal stress reduction based on univariate analysis. Our multivariate analysis further nuanced this observation, but again pointed to an overall association between hippocampal subfield changes and cortisol changes, but this time more at a systems level. Lastly, from a mechanistic viewpoint, we hypothesize that *Affect* training stimulates emotion-motivational (reward) systems associated with positive affect (79, 80), and regulated by oxytocin and opiates (110, 111). Since these neuropeptides are also involved in stress regulation (112, 113), they could be considered to provide a double hit, and prime candidates to mediate hippocampal volume increase and stress reduction in particular following compassion-based practice, yet also present following other practices.

It is of note that non-adherence to saliva sampling in ambulatory settings has been shown to exert a significant impact on the resulting cortisol data (114, 115) and that the present data does not fully conform to the recently provided consensus guidelines on the assessment of the CAR (116, 117), which were published after the conception of our study. Most importantly, we did not employ objective measures for the verification of participants' sampling times. Hence, diurnal cortisol data have to be treated with some caution since the possibility of non-adherence-related confounding cannot be excluded (114–117). We nevertheless addressed the issue of non-adherence through an experience sampling approach based on mobile phones handed out to our participants. As shown by the relatively low proportion of missing data, these devices may have boosted adherence by reminding participants of a forthcoming sampling time-point.

To conclude, using a longitudinal model, we investigated how different types of mental training differentially result in changes in hippocampal subfield volume, resting-state functional networks, and stress-related markers of diurnal cortisol and hair cortisol. We find that only the 3-months *Affect* training module cultivating compassion and care, rather than attention-based (*Presence*) or socio-cognitive (*Perspective*) training, related to an increase of hippocampal CA1-3 subfield volume, with corresponding alterations in functional connectivity and a reduction in total diurnal cortisol output. Across analyses we observed consistent alterations between cortisol change and CA1-3 volume and function, pinpointing this region as a potential target for further investigations on stress and the human brain. Lastly, our multivariate analyses also point to a circuit level understanding of latent diurnal stress scores. Our results may be informative for the development of targeted interventions to reduce stress, and inspire the update of models on the role of different hippocampal formations for human socio-emotional and stress-related processes.

Methods

The specifics on the experimental design are the similar to related works in the same sample (9, 118). They are provided again here for completeness.

Participants

We recruited a total of 332 healthy adults (197 women, mean±SD=40.7±9.2 years, 20-55 years), in the winters of 2012/2013 and 2013/2014. Participant eligibility was determined through a multi-stage procedure that involved several screening and mental health questionnaires, together with a phone interview [for details, see (71)]. Subsequently, a face-to-face mental health diagnostic interview with a trained clinical psychologist was carried out. The interview included a computer-assisted German version of the Structured Clinical Interview for DSM-IV Axis-I disorders, SCID-I DIA-X (118), and a personal interview, SCID-II, for Axis-II disorders (119, 120). Participants were excluded if they fulfilled criteria for: i) an Axis-I disorder within the past two years, ii) schizophrenia, psychotic disorders, bipolar disorder, or substance dependency, or iii) an Axis-II disorder at any time in their life. Participants taking medication influencing the HPA axis were also excluded. None of the participants had a history of suffering from neurological disorders or head trauma, based on an in-house self-report questionnaire completed prior to the neuroimaging investigations. Included participants furthermore underwent a diagnostic radiological evaluation to rule out the presence of mass lesions (e.g., tumors, vascular malformations). The study was approved by the Research Ethics Committees of University of Leipzig (#376/12-ff) and Humboldt University in Berlin (#2013-02, 2013-29, 2014-10), and all participants provided written informed consent prior to participation. The study was registered with the Protocol Registration System of ClinicalTrials.gov under the title “Plasticity of the Compassionate Brain” with the Identifier: NCT01833104. For more details on recruiting and sample selection, please see (71).

Sample size estimation and group allocation

Overall, 2595 people signed up for the *ReSource* study in winter 2012/2013. Of these individuals, 311 potential participants met all eligibility criteria. From the latter group, 198 were randomly selected as the final sample. Participants were selected from the larger pool of potential participants and assigned to cohorts using bootstrapping without replacement, creating cohorts that did not differ (omnibus test $p < 0.1$) in demographics (age, gender, marital status, income, and IQ) or self-reported traits (depression, empathy, interoceptive awareness, stress level, compassion for self and others, alexithymia, general mental health, anxiety, agreeableness, conscientiousness, extraversion, neuroticism, and openness). Seven participants dropped out of the study after assignment but before data collection began, leaving 30 participants in RCC1, 80 in TC1, and 81 in TC2.

2144 people applied for the second wave of the study in winter 2013/2014. Of these people, 248 potential participants met all the eligibility criteria. From the latter pool, 164 were then randomly selected as the final sample. Participants were selected from the larger pool of potential participants and assigned to cohorts using bootstrapping without replacement, creating cohorts that did not differ significantly (omnibus test, $p > 0.1$) from the Winter 2012/2013 cohorts or from one another in demographics (age, gender, marital status, income, and IQ) or self-reported traits (depression, empathy, interoceptive awareness, stress level, compassion for self and others, alexithymia, general mental health, anxiety, agreeableness, conscientiousness, neuroticism, and openness). The control cohorts (RCC1, RCC2, and RCC1&2) were significantly lower in extraversion than TC3; participants in the control cohorts were also more likely to have children than participants in TC3. Twenty-three participants dropped out of the study after assignment but before data collection began, leaving 81 participants in TC3 and 60 in RCC2. See further (71 [↗](#)).

ReSource training program

In the *ReSource Project*, we investigated the specific effects of commonly used mental training techniques by parceling the training program into three separate modules (*Presence*, *Affect* and *Perspective*). Participants were selected from a larger pool of potential volunteers by bootstrapping without replacement, creating cohorts not differing significantly with respect to several demographic and self-report traits (71 [↗](#)). Each cultivated distinct cognitive and socio-affective capacities (69 [↗](#)). Participants were divided in two 9-month training cohorts experiencing the modules in different orders, one 3-month *Affect* training cohort and one retest control cohort. In detail, two training cohorts (TC1, TC2) started their training with the mindfulness-based *Presence* module. They then underwent *Affect* and *Perspective* modules in different orders thereby acting as mutual active control groups. To isolate the specific effects of the *Presence* module, a third training cohort (TC3) underwent the 3-month *Affect* module only (Fig. 1B [↗](#)).

As illustrated in Fig 1A [↗](#), the core psychological processes targeted in the *Presence* module are attention and interoceptive awareness, which are trained through the two meditation-based core exercises Breathing Meditation and Body Scan. The *Affect* module targets the cultivation of social emotions such as compassion, loving kindness and gratitude. It also aims to enhance prosocial motivation and dealing with difficult emotions. The two core exercises of the *Affect* module are Loving-kindness Meditation and Affect Dyad. In the *Perspective* module participants train meta-cognition and perspective-taking on self and others through the two core exercises Observing-thoughts Meditation and Perspective Dyad. The distinction between *Affect* and *Perspective* modules reflects research identifying distinct neural routes to social understanding: One socio-affective route including emotions such as empathy and compassion, and one socio-cognitive route including the capacity to mentalize and take perspective on self and others (for details on the scientific backbone of this division see: (71 [↗](#))).

The two contemplative dyads are partner exercises that were developed for the *ReSource* training (121 [↗](#)). They address different skills such as perspective taking on self and others (*Perspective* dyad) or gratitude, acceptance of difficult emotions and empathic listening (*Affect* dyad), but are similar in structure (for details see: (71 [↗](#))). In each 10-min dyadic practice, two randomly paired participants share their experiences with alternating roles of speaker and listener. The dyadic format is designed to foster interconnectedness by providing opportunities for self-disclosure and non-judgmental listening (71 [↗](#), 121 [↗](#)). Our recommendation was to train for a minimum of 30 minutes (e.g. 10 minutes contemplative dyad, 20 minutes classic meditation) on five days per week.

MRI acquisition

MRI data were acquired on a 3T Siemens Magnetom Verio (Siemens Healthcare, Erlangen, Germany) using a 32-channel head coil. Structural images were acquired using a T1-weighted 3D-MPRAGE sequence (repetition time [TR]=2300 ms, echo time [TE]=2.98 ms, inversion time [TI]=900

ms, flip angle=7°; 176 sagittal slices with 1mm slice thickness, field of view [FOV]=240×256 mm², matrix=240×256, 1×1×1 mm³ voxels). We recorded task-free functional MRI using a T2*-weighted gradient EPI sequence (TR=2000ms, TE=27ms, flip angle=90°; 37 slices tilted at approximately 30° with 3 mm slice thickness, FOV=210×210mm², matrix=70×70, 3×3×3 mm³ voxels, 1 mm gap; 210 volumes per session). During the functional session, participants were instructed to lie still in the scanner, think of nothing in particular, and fixate a white cross in the center of a black screen.

Structural MRI analysis: Hippocampal subfield volumetry

Based on the available high-resolution T1-weighted images subiculum (SUB), CA1-3, and CA4/DG were segmented using a patch-based algorithm in all participants individually (see further (74)). Shortly, this procedure uses a population-based patch normalization relative to a template library (122), providing good time and space complexity. In previous validations work, this algorithm has shown high segmentation accuracy of hippocampal subfields (74), and in detecting hippocampal subfield pathology in patients with epilepsy (91). It was furthermore demonstrated that these representations can be used to probe sub-regional functional organization of the hippocampus (35, 36). Hippocampal volumes were estimated based on T1w data that were linearly registered to MNI152 using FSL flirt (<http://www.fmrib.ox.ac.uk/fsl/>), such that intracranial volume was implicitly controlled for.

As previously reported (123), for successful hippocampus segmentations, an initial quality check was conducted by two independent raters, R.L. and L.P. Both raters were blind to participant characteristics including age, sex, and training or control group. In short, each segmentation was rated for quality on a scale of 1–10, with points being subtracted depending on the severity of detected flaws. One point was subtracted for minor flaws, e.g. part of a segmentation extends slightly beyond the hippocampal boundary, or does not cover a small aspect of the hippocampal formation. Two points were subtracted for medium flaws, e.g. gaps between sub-field segmentations. Finally, major flaws immediately qualified for resampling, and included e.g. one or more subfield segmentations being clearly misplaced. Given a minimum of 70% inter-rater reliability, segmentation ratings were then averaged and evaluated, with scores of 5 and lower qualifying for reprocessing with the algorithm. Following this second round of processing, segmentations were rated again. Any remaining segmentations with average scores lower than 5 were excluded from the analysis.

Task-free functional MRI analysis: Hippocampal connectivity

Processing was based on DPARSF/REST for Matlab (<http://www.restfmri.net> (124)). We discarded the first 5 volumes to ensure steady-state magnetization, performed slice-time correction, motion correction and realignment, and co-registered functional time series of a given subject to the corresponding T1-weighted MRI. Images underwent unified segmentation and registration to MNI152, followed by nuisance covariate regression to remove effects of average WM and CSF signal, as well as 6 motion parameters (3 translations, 3 rotations). We included a *scrubbing* (125) that modeled time points with a frame-wise displacement of ≥0.5 mm, together with the preceding and subsequent time points as separate regressors during nuisance covariate correction.

We linearly co-registered the extracted hippocampal subfield volumes with the functional MRI data for each individual using FSL flirt (<http://www.fmrib.ox.ac.uk/fsl/>), followed by nearest neighbor interpolation. Following, we generated functional connectivity maps from both the left and right hippocampal subfields in each individual. Functional connectivity was calculated as the correlation between the mean time series of the seed region and the time series of all cortical parcels based on the Schaefer 400 parcellation. To render them normally distributed and scale the profiles across participants, correlation coefficients underwent a Fisher r-to-z transformation and were rescaled, resulting in connectivity profiles between 0 and 1 for each participant and

timepoint. Functional networks were defined as the top 10% regions based on mean connectivity profile of the respective subfield in the ipsilateral hemisphere at baseline. Individuals with a framewise-displacement of $>0.3\text{mm}$ ($<5\%$) were excluded.

Diurnal cortisol assessments

For cortisol assessment, 14 saliva samples (7 per day) were obtained over the course of two consecutive weekdays (Mondays/Tuesdays, Wednesdays/Thursdays or Thursdays/Fridays, depending on participant availability). In detail, samples were taken upon free awakening (while still in bed; S1) and at 30 minutes, 60 minutes, 4, 6, 8 and 10 hours after awakening. Saliva was collected using Salivette collection devices (Sarstedt, Nuembrecht, Germany). Participants were instructed to place collection swabs in their mouths and to refrain from chewing for 2 minutes. They were asked to not eat, drink (except water), or brush their teeth during the 10 minutes before sampling, and to not smoke during the 30 minutes before sampling. If deviating from this guideline, they were asked to thoroughly rinse their mouth with water before taking a sample. Participants otherwise followed their normal daily routine. To maximize adherence to the sampling protocol, participants were given pre-programmed mobile devices using an inhouse application that reminded them to take each (except the first) Salivette at the designated time. Sampling times of the non-morning probes were jittered (± 15 min) to avoid complete predictability. Samples were kept in the freezer until returned to the laboratory, where they were stored at -30°C until assay (at the Department of Biological and Clinical Psychology, University of Trier, Germany). Cortisol levels (expressed in nmol/l) were determined using a time-resolved fluorescence immunoassay (126) with intra-/inter-assay variability of 10/12%.

Raw cortisol data were each treated with a natural log transformation to remedy skewed distributions. Across the full sample, any values diverging more than 3 SD from the mean were labeled outliers and winsorized to the respective upper or lower 3 SD boundary to avoid influential cases. Logged and winsorized cortisol data was then averaged across the two sampling days, and the most commonly used summary indices of diurnal cortisol activity were calculated (13). The CAR was quantified as a change score from S1 to either the 30-or 60-minute post-awakening sample, depending on the individual peak in hormone levels. If participants peaked at S1 rather than at 30 or 60 minutes thereafter, the 30-minute data point was used to operationalize the (inverse) CAR, given that it was always closer in magnitude to S1 than the 60-minute data point. The cortisol decline over the course of the day (diurnal slope) was operationalized as a change score from baseline to the final sample of the day (at 600 minutes after awakening). Total daily cortisol output was operationalized as the area under the curve with respect to ground, AUC_g (127), which considers the difference between the measurements from each other (i.e., the change over time) and the distance of these measures from zero (i.e., the level at which the change over time occurs). Awakening, 240, 360, 480, and 600 minutes post-awakening cortisol values were included in the calculation of the AUC_g . To prevent it from having an undue influence, the CAR samples at 30 and 60 minutes were excluded from the total output score calculation. On each sampling day, awakening time and sleep duration were registered using the pre-programmed mobile device immediately upon awakening in parallel to taking the first Salivette. These measures were averaged across the two sampling days to minimize situational influences.

Assay of Steroid Hormone Concentration in Hair

Please see further details on sample and dropout in (5). To evaluate cortisol and cortisone, hair strands were taken as close as possible to the scalp from a posterior vertex position at T0 and after each following timepoint (T0-T3). Hair samples were enfolded in aluminum foil and stored in the dark at room temperature until assay at the Department of Psychology, TU Dresden, Germany. We evaluated the proximal 3-cm segment of hair to study accumulation of cortisol and cortisone over each 3-month period, based on the assumption of an average hair growth rate of 1 cm/month (128). Hormone concentrations were captured using liquid chromatography–tandem mass spectrometry, the current criterion standard approach for hair steroid analysis (129). All

hormone concentrations were reported in picograms per milligram. For the current longitudinal research aim, all samples of one participant were always run with the same reagent batch to avoid intraindividual variance due to batch effects.

Quality control and case selection

Structural MRI data without artifacts and acceptable automated segmentations were available in 943 participants. Functional MRI data were available in 849 participants. We opted to have consistent sample sizes in structure and function and therefor including only people that had both structural and functional data available. Please see **Table 4.** for participant numbers across timepoints and measures for structural and functional data.

	Structural MRI data	Structural and Functional MRI data
T0	288 (TC3:71)	258 (TC3: 70)
T1	272 (TC3:68)	238 (TC3: 64)
T2	193	172
T3	190	181

Table 6.

Sample size per timepoint.

Reason for dropout	T ₀	T ₁	T ₂	T ₃
(TC1, TC2, RCC: N=251)				
Structural MR incidental finding	5	(5 based on T ₀)	(5 based on T ₀)	(5 based on T ₀)
Structural MRI quality control	7	6	4	2
Dropout	2	7(2 based on T ₀)	9(7 based on T ₀₁)	16(9 based on T ₀₁₂)
Medical reasons	1	7(1 based on T ₀)	8(7 based on T ₀₁)	15(8 based on T ₀₁₂)
Other	4	10	7	7
Functional MRI missing/low QC	29	30	21	9
Hippocampal QC	15	12	25	16

Table 7.

Reason for missing data across the study duration.

MR incidental findings are based on T₀ radiological evaluations; participants who did not meet *MRI quality control* criteria refers to movement and/or artefacts in the T1-weighted MRI; dropout details can be found in (71); *no MRT*: due to illness / scheduling issues / discomfort in scanner; *other*: non-disclosed; *functional MRI missing*: no complete functional MRI; *functional MRI quality*: >0.3mm movement (low quality in volume + surface)

Table 8.

Reason for missing data across the study duration.

MR incidental findings are based on T_0 radiological evaluations; participants who did not survive *MRI quality control* refers to movement and/or artefacts in the T1-weighted MRI; dropout details can be found in (71); *no MRT*: due to illness / scheduling issues / discomfort in scanner; *other*: non-disclosed.

Among those, salivary cortisol measures were available in *Presence* $n = 85$ (53 females, age = 40.87 std 9.69, 20-55), *Affect* $n = 89$ (50 females, age = 40.11 std 9.87, 20-55), *Perspective* $n = 81$ (48 females, age = 40.14 std 9.78, 20-55). Hair cortisol change scores were available in *Presence* $n = 31$ (21 females, age = 39.55 std 10.40, 20-54), *Affect* $n = 44$ (24 females, age = 37.52 std 10.78, 20-54), *Perspective* $n = 41$ (24 females, age = 38.14 std 10.51, 20-54).

Statistical analyses

Using SurfStat (130, 131), we carried out structural and functional MRI analysis for the left and right hippocampal subfield difference scores between different 3-month timepoints. All models statistically corrected for nuisance effects of age and sex, as well as random effect of subject. Main contrasts considered in the group analyses concern *Presence* versus *Active Control* (T_0 - T_1) and *Affect* versus *Perspective* (T_1 - T_3). Additionally, investigations include analyses versus Retest Control Cohort as well as subgroups defined by training cohort and timepoint. In case of multiple comparison, we performed Bonferroni correction (132).

Partial least squares analysis

To assess potential relationships between cortisol change and hippocampal subfield volume and functional change, we performed a partial least squares analysis (PLS) (77, 78). PLS is a multivariate associative model that optimizes the covariance between two matrices, by generating latent components (LCs), which are optimal linear combinations of the original matrices (77, 78). In our study, we utilized PLS to analyze the relationships between change in volume and intrinsic function of hippocampal subfields and diurnal cortisol measures. Here we included all Training Modules and regressed out effects of age, sex, and random effects of subject on the brain measures before conducting the PLS analysis. The PLS process involves data normalization within training groups, cross-covariance, and singular value decomposition.

Subsequently, subfield and behavioral scores are computed, and permutation testing (1000 iterations) is conducted to evaluate the significance of each latent factor solution (FDR corrected). We report then the correlation of the individual hippocampal and cortisol markers with the latent factors. To estimate confidence intervals for these correlations, we applied a bootstrapping procedure that generated 100 samples with replacement from subjects' RSFC and behavioral data.

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Author contributions

SLV and BCB were involved in data acquisition and processing of MRI data, and conceived and designed the MRI-based experiments. VE was involved in data acquisition and processing of diurnal cortisol data, and designed the cortisol-based experiments, LP and RL helped with the quality control of the hippocampal data. BC, AB and NB designed the hippocampal segmentation protocol. T.S. initiated and developed the *ReSource* Project and model, as well as the training protocol. All authors discussed, wrote, and approved the final version of the manuscript.

Competing interests

The authors declare that they have no competing interests.

Data and code availability

In line with EU data regulations (General Data Protection Regulation, GDPR), we regret that data cannot be shared publicly because we did not obtain explicit participant agreement for data-sharing with third parties. Our work is based on personal data (age, sex and neuroimaging data) that could be matched to individuals. The data is therefore pseudonymized rather than anonymized and falls under the GDPR. Data are available upon request (contact via valk@cbs.mpg.de). Summary data and analysis scripts (Matlab) to reproduce primary analyses and figures are publicly available on GitHub (https://github.com/CNG-LAB/valk_hippocampal_change ) and raw data-plots are provided whenever possible.

Supplementary analyses

Supplementary File 1a. Descriptive statistics T0-T1, p-values are uncorrected, q values reported when $p < .05$.

Presence	LSUB	LCA1-3	LCA4/DG	RSUB	RCA1-3	RCA4/DG
<i>t-values</i>	1,335	0,836	-0,452	-0,623	0,788	-0,751
<i>p-value</i>	0,183	0,404	0,652	0,534	0,432	0,454
<i>Mean</i>	4,616	30,930	-4,686	-7,279	13,244	-2,419
<i>std</i>	83,141	205,390	51,976	98,493	166,340	64,548
<i>CI min</i>	-13,209	-13,105	-15,830	-28,396	-22,419	-16,258
<i>CI max</i>	22,442	74,965	6,458	13,838	48,907	11,421
Affect TC3						
<i>t-values</i>	1,195	1,456	1,739	1,528	1,334	0,060
<i>p-value</i>	0,234	0,147	0,084	0,128	0,184	0,952
<i>Mean</i>	4,295	46,443	7,312	19,426	23,344	3,705
<i>std</i>	90,258	206,540	43,280	121,990	187,470	63,729
<i>CI min</i>	-18,821	-6,454	-3,773	-11,816	-24,670	-12,617
<i>CI max</i>	27,411	99,340	18,396	50,669	71,358	20,027
RCC						
<i>t-values</i>	-0,955	-0,497	-1,698	-0,126	-2,526	0,778
<i>p-value</i>	0,341	0,620	0,091	0,900	0,012, q=0.072	0,437
<i>Mean</i>	-14,000	6,259	-12,111	-0,870	-50,259	10,407
<i>std</i>	66,176	156,220	41,762	91,759	139,760	79,943
<i>CI min</i>	-32,063	-36,381	-23,510	-25,916	-88,406	-11,413
<i>CI max</i>	4,063	48,900	-0,712	24,175	-12,112	32,228

Supplementary File 1b. Descriptive statistics T1-T3, p-values are uncorrected, q values reported when $p < .05$.

Perspective	LSUB	LCA1-3	LCA4/DG	RSUB	RCA1-3	RCA4/DG
<i>t-values</i>	0,456	-1,143	-0,463	0,573	-2,118	1,291
<i>p-value</i>	0,649	0,254	0,644	0,567	0,035, q>0.1	0,198
<i>Mean</i>	4,434	-23,048	-1,398	2,108	-39,602	12,024
<i>std</i>	71,215	137,810	45,224	99,892	208,470	76,355
<i>CI min</i>	-11,116	-53,139	-11,273	-19,704	-85,122	-4,649
<i>CI max</i>	19,984	7,043	8,477	23,921	5,917	28,697
Affect						
<i>t-values</i>	1,121	2,495	0,235	0,210	2,374	0,394
<i>p-value</i>	0,263	0,013, q=0.078	0,814	0,833	0,018, q>0.1	0,694
<i>Mean</i>	8,424	25,511	1,489	-2,098	40,120	5,087
<i>std</i>	63,328	130,470	36,293	112,520	181,300	76,124
<i>CI min</i>	-4,691	-1,509	-6,027	-25,399	2,573	-10,678
<i>CI max</i>	21,539	52,531	9,005	21,204	77,666	20,852
RCC						
<i>t-values</i>	-1,102	-1,118	-1,052	-0,409	1,052	-0,557

<i>p-value</i>	0,271	0,264	0,294	0,683	0,294	0,578
<i>Mean</i>	-6,864	-21,845	-3,700	-8,591	15,673	-1,827
<i>std</i>	75,284	137,630	45,632	104,680	155,170	71,276
<i>CI min</i>	-21,090	-47,853	-12,323	-28,372	-13,650	-15,297
<i>CI max</i>	7,363	4,162	4,923	11,190	44,995	11,642

Supplementary File 1c. T0-T1 change statistics, p-values are uncorrected, q values reported when $p < .05$.

Affect TC3 vs Presence	LSUB	LCA1-3	LCA4/DG	RSUB	RCA1-3	RCA4/DG
<i>t-value</i>	-0,065	0,454	1,519	1,487	0,401	0,548
<i>p-value</i>	0,948	0,650	0,130	0,139	0,689	0,584
<i>Cohens D</i>	-0,009	0,065	0,217	0,212	0,057	0,078
Affect TC3 vs RCC						
<i>t-value</i>	1,359	1,228	2,175	1,036	2,452	-0,461
<i>p-value</i>	0,176	0,221	0,031, q>0.1	0,302	0,015, q=0.09	0,645
<i>Cohens D</i>	0,194	0,175	0,311	0,148	0,350	-0,066
Presence vs RCC						
<i>t-value</i>	1,522	0,883	0,875	-0,317	2,248	-1,021
<i>p-value</i>	0,130	0,379	0,383	0,752	0,026, q>0.1	0,308
<i>Cohens D</i>	0,217	0,126	0,125	-0,045	0,321	-0,146

Supplementary File 1d. T1-T3 change statistics, p-values are uncorrected, q values reported when $p < .05$.

Affect vs Perspective	LSUB	LCA1-3	LCA4/DG	RSUB	RCA1-3	RCA4/DG
<i>t-value</i>	0,417	2,360	0,458	-0,245	2,930	-0,604
<i>p-value</i>	0,677	0,019, q>0.1	0,647	0,807	0,004, q=0.022	0,547
<i>Cohens D</i>	0,050	0,282	0,055	-0,029	0,350	-0,072
Affect vs RCC						
<i>t-value</i>	1,504	2,460	0,861	0,417	0,935	0,641
<i>p-value</i>	0,134	0,014, q=0.084	0,390	0,677	0,351	0,522
<i>Cohens D</i>	0,180	0,294	0,103	0,050	0,112	0,077
Perspective vs RCC						
<i>t-value</i>	1,025	-0,067	0,359	0,659	-2,139	1,250
<i>p-value</i>	0,306	0,947	0,720	0,510	0,033, q>0.1	0,212
<i>Cohens D</i>	0,123	-0,008	0,043	0,079	-0,256	0,149

Supplementary File 1e. T1-T3 change statistics – Training cohort 1 and 2 *Affect* versus *Perspective*. P-values are uncorrected, q values reported when $p < .05$.

TC1	LSUB	LCA1-3	LCA4/DG	RSUB	RCA1-3	RCA4/DG
<i>t-value</i>	1,557	2,549	1,263	0,214	4,243	-0,224
<i>p-value</i>	0,122	0,012, q=0.072	0,209	0,831	0,000 q<0.001	0,823
<i>Cohens D</i>	0,273	0,447	0,222	0,038	0,744	-0,039
TC2						
<i>t-value</i>	-0,819	0,447	-0,300	-0,757	0,102	-0,681
<i>p-value</i>	0,414	0,656	0,765	0,451	0,919	0,497
<i>Cohens D</i>	-0,149	0,081	-0,055	-0,138	0,019	-0,124

Supplementary File 1f. T1-T2 change. P-values are uncorrected, q values reported when $p < .05$.

T1-T2 Affect vs Perspective	LSUB	LCA1-3	LCA4/DG	RSUB	RCA1-3	RCA4/DG
<i>t-value</i>	0,612	1,751	0,944	0,404	3,743	-0,174
<i>p-value</i>	0,541	0,082	0,347	0,687	0,000 q<0.001	0,862
<i>Cohens D</i>	0,108	0,308	0,166	0,071	0,659	-0,031
Affect vs RCC						
<i>t-value</i>	1,995	0,967	0,582	0,974	2,637	0,622
<i>p-value</i>	0,048 q>0.1	0,335	0,562	0,332	0,009 q=0.054	0,535
<i>Cohens D</i>	0,351	0,170	0,102	0,171	0,464	0,110
Perspective vs RCC						
<i>t-value</i>	1,271	-0,938	-0,448	0,508	-1,454	0,786
<i>p-value</i>	0,206	0,350	0,655	0,613	0,148	0,433
<i>Cohens D</i>	0,224	-0,165	-0,079	0,089	-0,256	0,138

Supplementary File 1g. T2-T3 change. P-values are uncorrected, q values reported when $p < .05$.

T2-T3 Affect vs Perspective	LSUB	LCA1-3	LCA4/DG	RSUB	RCA1-3	RCA4/DG
<i>t-value</i>	-0,033	1,768	-0,176	-0,582	0,641	-0,748
<i>p-value</i>	0,974	0,079	0,860	0,561	0,523	0,456
<i>Cohens D</i>	-0,005	0,293	-0,029	-0,096	0,106	-0,124
Affect vs RCC						
<i>t-value</i>	0,212	2,691	0,832	-0,302	-1,006	0,326
<i>p-value</i>	0,832	0,008, q=0.048	0,407	0,763	0,316	0,745
<i>Cohens D</i>	0,035	0,445	0,138	-0,050	-0,167	0,054
Perspective vs RCC						
<i>t-value</i>	0,239	0,828	0,988	0,296	-1,628	1,075
<i>p-value</i>	0,811	0,409	0,325	0,768	0,106	0,284
<i>Cohens D</i>	0,040	0,137	0,164	0,049	-0,269	0,178

Supplementary File 1h. Subfield-specific changes following the Training Modules, controlling for the other two ipsilateral subfields

	LSUB	LCA1-3	LCA4/DG	RSUB	RCA1-3	RCA4/DG
<i>Presence vs Active Control</i>	t=0.475 p>0.1 D=0.069	t=-0.033 p>0.1 D=-0.004	t=-1.514 p>0.1 D=-0.216	t=-1.365 p>0.1 D=-0.195	t=-0.162 p>0.1 D=-0.023	t=-0.210 p>0.1 D=-0.03
<i>Affect vs Perspective</i>	t=0.793 p>0.1 D=-0.03	t=2.298, p=0.022 D=0.275	t=0.924 p>0.1 D=-0.012	t=0.883 p>0.1 D=-0.018	t=3.045 p=0.0025 Q=0.015 D=0.364	t=-0.978 p>0.1 D=-0.117

Supplementary File 1i. Overall change in subfield volume.

Training vs RCC	LSUB	LCA1-3	LCA4/DG	RSUB	RCA1-3	RCA4/DG
<i>t-value</i>	1,033	0,702	1,035	-1,348	0,774	0,017
<i>p-value</i>	0,303	0,484	0,302	0,180	0,440	0,986
<i>Cohens D</i>	0,172	0,117	0,172	-0,225	0,129	0,003

Supplementary File 1j. Sex differences (female versus male) in hippocampal subfield volumes.

	LSUB	LCA1-3	LCA4/DG	RSUB	RCA1-3	RCA4/DG
baseline	t=2.423 p=0.008 q=0.049	t=2.061 p=0.021 q=0.121	t=3.671 p<0.001 q=0.001	t=-0.520 p=0.698 q>0.05	t=3.570 p<0.001 q>0.002	t=2.555 p=0.006 q=0.034
baseline+ ICV control	t=1.739 p=0.042 q>0.1	t=-0.171 p>0.1 q>0.1	t=1.259 p>0.1 q>0.1	t=-0.358 p>0.1 q>0.1	t=1.962 p=0.025 q>0.1	t=1.201 p>0.1 q>0.1
Main change analysis						
<i>Affect versus Perspective ICV control</i>	t=0.514 p>0.05 q>0.05	t=2.588 p=0.01 q=0.06	t=0.677 p>0.05 q>0.05	t=-0.262 p>0.05 q>0.05	t=3.065 p=0.002 q=0.014	t=-0.511 p>0.05 q>0.05
<i>Presence versus Active Control ICV control</i>	t=-0.101 p>0.05 q>0.05	t=0.214 p>0.05 q>0.05	t=1.236 p>0.05 q>0.05	t=1.462 p>0.05 q>0.05	t=0.256 p>0.05 q>0.05	t=0.406 p>0.05 q>0.05

Supplementary File 1k. Descriptive statistics mean subfield functional network change T0-T1. Main focus was on CA1-3 based on volumetric observations and are highlighted in bold. For these multiple comparisons (FDRq, corrected for two subfields) values are reported if uncorrected p values are below p<.05.

Presence	LSUB	LCA1-3	LCA4/DG	RSUB	RCA1-3	RCA4/DG
<i>t-values</i>	-1,223	-0,803	-0,915	-0,757	-0,507	0,387
<i>p-value</i>	0,223	0,423	0,361	0,450	0,613	0,699
<i>Mean</i>	-0,004	-0,001	-0,008	0,000	0,009	0,004
<i>std</i>	0,091	0,089	0,093	0,089	0,099	0,087
<i>CI min</i>	-0,023	-0,020	-0,028	-0,018	-0,012	-0,014
<i>CI max</i>	0,015	0,018	0,011	0,019	0,029	0,023
Affect TC3						
<i>t-values</i>	-1,089	-0,247	-0,105	-1,053	-1,071	0,587
<i>p-value</i>	0,278	0,805	0,917	0,294	0,285	0,558
<i>Mean</i>	-0,004	0,003	0,000	-0,004	0,002	0,007
<i>std</i>	0,078	0,091	0,082	0,085	0,081	0,096
<i>CI min</i>	-0,024	-0,021	-0,021	-0,026	-0,019	-0,018
<i>CI max</i>	0,016	0,026	0,021	0,018	0,022	0,032
RCC						
<i>t-values</i>	-0,521	-1,462	0,023	0,198	1,384	0,296
<i>p-value</i>	0,603	0,145	0,981	0,843	0,168	0,767
<i>Mean</i>	-0,001	-0,011	0,000	0,009	0,029	0,004
<i>std</i>	0,107	0,095	0,100	0,094	0,097	0,080
<i>CI min</i>	-0,030	-0,036	-0,028	-0,017	0,002	-0,017
<i>CI max</i>	0,028	0,015	0,027	0,034	0,055	0,026

Supplementary File 1l. Descriptive statistics mean subfield functional network change T1-T3. Main focus was on CA1-3 based on volumetric observations and are highlighted in bold. For these multiple comparisons (FDRq, corrected for two subfields) values are reported if uncorrected p values are below p<.05.

Perspective	LSUB	LCA1-3	LCA4/DG	RSUB	RCA1-3	RCA4/DG
<i>t-value</i>	0,046	-0,071	0,461	-1,443	-2,012	-1,089
<i>p-value</i>	0,963	0,943	0,645	0,150	0,045, q>0.1	0,277
<i>Mean</i>	-0,003	-0,007	0,003	-0,012	-0,024	-0,006
<i>std</i>	0,088	0,100	0,092	0,089	0,081	0,099
<i>CI min</i>	-0,022	-0,029	-0,017	-0,032	-0,041	-0,028
<i>CI max</i>	0,017	0,015	0,023	0,007	-0,006	0,016
Affect						
<i>t-values</i>	1,050	0,139	0,899	1,116	1,691	0,569
<i>p-value</i>	0,295	0,889	0,369	0,265	0,092	0,570
<i>Mean</i>	0,007	-0,005	0,007	0,011	0,010	0,010
<i>std</i>	0,103	0,110	0,100	0,092	0,098	0,098
<i>CI min</i>	-0,015	-0,028	-0,013	-0,008	-0,010	-0,010
<i>CI max</i>	0,028	0,018	0,028	0,030	0,031	0,030
RCC						
<i>t-values</i>	0,225	1,206	-0,608	-0,839	-0,701	-0,436
<i>p-value</i>	0,822	0,229	0,544	0,402	0,484	0,663
<i>Mean</i>	0,000	0,005	-0,005	-0,006	-0,010	0,001
<i>std</i>	0,079	0,079	0,071	0,089	0,092	0,092
<i>CI min</i>	-0,015	-0,010	-0,019	-0,023	-0,028	-0,017
<i>CI max</i>	0,014	0,020	0,008	0,011	0,007	0,018

Supplementary File 1m. Functional connectivity network change T0-T1. Main focus was on CA1-3 based on volumetric observations and are highlighted in bold. For these multiple comparisons (FDRq, corrected for two subfields) values are reported if uncorrected p values are below p<.05.

Affect TC3 vs Presence

	LSUB	LCA1-3	LCA4/DG	RSUB	RCA1-3	RCA4/DG
<i>t-value</i>	0,058	0,366	0,541	-0,230	-0,411	0,151
<i>p-value</i>	0,953	0,715	0,589	0,818	0,682	0,880
<i>Cohens D</i>	0,008	0,052	0,077	-0,033	-0,058	0,021
Affect TC3 vs RCC						

<i>t-value</i>	-0,347	0,782	-0,080	-0,785	-1,556	0,177
<i>p-value</i>	0,729	0,435	0,936	0,433	0,121	0,860
<i>Cohens D</i>	-0,049	0,111	-0,011	-0,112	-0,221	0,025
Presence vs RCC						
<i>t-value</i>	-0,430	0,491	-0,607	-0,626	-1,283	0,046
<i>p-value</i>	0,668	0,624	0,545	0,532	0,201	0,964
<i>Cohens D</i>	-0,061	0,070	-0,086	-0,089	-0,182	0,006

Supplementary File 1n. Functional connectivity network change T1-T3. Main focus was on CA1-3 based on volumetric observations and are highlighted in bold. For these multiple comparisons (FDRq, corrected for two subfields) values are reported if uncorrected p values are below p<.05.

Affect vs Perspective

	LSUB	LCA1-3	LCA4/DG	RSUB	RCA1-3	RCA4/DG
<i>t-value</i>	0,644	0,137	0,272	1,674	2,420	1,088
<i>p-value</i>	0,520	0,891	0,786	0,095	0,016 q=0.032	0,278
<i>Cohens D</i>	0,077	0,016	0,032	0,200	0,289	0,130
Affect vs RCC						
<i>t-value</i>	0,575	-0,703	1,023	1,326	1,631	0,681
<i>p-value</i>	0,566	0,483	0,307	0,186	0,104	0,496
<i>Cohens D</i>	-0,049	0,111	-0,011	-0,112	-0,221	0,025
Perspective vs RCC						
<i>t-value</i>	-0,113	-0,824	0,709	-0,458	-0,939	-0,472
<i>p-value</i>	0,910	0,410	0,479	0,648	0,348	0,637
<i>Cohens D</i>	-0,061	0,070	-0,086	-0,089	-0,182	0,006

Supplementary File 1o. Functional connectivity network change T1-T3: Training cohort 1 and 2 *Affect versus Perspective*. Main focus was on CA1-3 based on volumetric observations and are highlighted in bold. For these multiple comparisons (FDRq, corrected for two subfields) values are reported if uncorrected p values are below p<.05. Explorative analyses take all 6 subfields into account.

TC1	LSUB	LCA1-3	LCA4/DG	RSUB	RCA1-3	RCA4/DG
<i>t-value</i>	-0,362	-0,985	-0,339	-0,254	-0,417	-0,085
<i>p-value</i>	0,718	0,326	0,735	0,800	0,677	0,933
<i>Cohens D</i>	-0,063	-0,172	-0,059	-0,044	-0,073	-0,015
TC2						
<i>t-value</i>	1,189	1,171	0,669	2,873	3,815	1,683
<i>p-value</i>	0,237	0,244	0,505	0,005 q=0.03 (6)	0,000 q<0.001	0,095
<i>Cohens D</i>	0,215	0,212	0,121	0,520	0,691	0,305

Supplementary File 1p. Functional connectivity network change T1-T2. Main focus was on CA1-3 based on volumetric observations and are highlighted in bold. For these multiple comparisons (FDRq, corrected for two subfields) values are reported if uncorrected p values are below p<.05.

Affect vs Perspective	LSUB	LCA1-3	LCA4/DG	RSUB	RCA1-3	RCA4/DG
<i>t-value</i>	-0,423	0,055	-0,713	0,810	2,121	0,737
<i>p-value</i>	0,673	0,956	0,477	0,419	0,036 q=0.072	0,463
<i>Cohens D</i>	-0,074	0,010	-0,126	0,143	0,373	0,130
Affect vs RCC						
<i>t-value</i>	-0,766	-1,978	-0,285	0,088	0,923	-0,061
<i>p-value</i>	0,445	0,051	0,776	0,930	0,358	0,952
<i>Cohens D</i>	-0,049	0,111	-0,011	-0,112	-0,221	0,025
Perspective vs RCC						
<i>t-value</i>	-0,287	-1,967	0,487	-0,780	-1,375	-0,846
<i>p-value</i>	0,775	0,052	0,627	0,437	0,172	0,399
<i>Cohens D</i>	-0,061	0,070	-0,086	-0,089	-0,182	0,006

Supplementary File 1q. Functional connectivity network change T2-T3. Main focus was on CA1-3 based on volumetric observations and are highlighted in bold. For these multiple comparisons (FDRq, corrected for two subfields) values are reported if uncorrected p values are below p<.05.

Affect vs Perspective	LSUB	LCA1-3	LCA4/DG	RSUB	RCA1-3	RCA4/DG
<i>t-value</i>	1,378	0,202	1,037	1,678	1,501	0,911
<i>p-value</i>	0,170	0,840	0,301	0,096	0,136	0,364
<i>Cohens D</i>	0,227	0,033	0,171	0,277	0,248	0,150
Affect vs RCC						
<i>t-value</i>	1,488	0,760	1,598	1,630	1,192	0,996
<i>p-value</i>	0,139	0,449	0,112	0,105	0,235	0,321
<i>Cohens D</i>	-0,049	0,111	-0,011	-0,112	-0,221	0,025
Perspective vs RCC						
<i>t-value</i>	0,045	0,533	0,499	-0,121	-0,367	0,043
<i>p-value</i>	0,964	0,595	0,619	0,904	0,714	0,966
<i>Cohens D</i>	-0,061	0,070	-0,086	-0,089	-0,182	0,006

Supplementary File 1r. Correlating change in subfield volume and diurnal cortisol indices in Affect. Main focus was on CA1-3 based on volumetric observations and are highlighted in bold. For these multiple comparisons (FDRq, corrected for two subfields) values are reported if uncorrected p values are below p<.05.

	LSUB	LCA1-3	LCA4/DG	RSUB	RCA1-3	RCA4/DG
CAR	1,007, p>0.1	-0,355, p>0.1	0,166, p>0.1	-1,364, p>0.1	-1,543, p>0.1	-0,404, p>0.1
Slope	-0,283, p>0.1	-0,878, p>0.1	0,728, p>0.1	0,634, p>0.1	-1,245, p>0.1	-1,716, p<0.1
AUC _g	-0,945, p>0.1	-2,237, p=0.028, q=0.056	0,636, p>0.1	-0,222, p>0.1	-2,283, p=0.025, q=0.05	-1,446, p>0.1

Supplementary File 1s. Association between stress-markers and within functional network sub-regions in *Affect and Perspective*.

	<i>Affect</i>	LCA1-3 - PI	RCA1-3 - mPFC	<i>Perspective</i>	LCA1-3 - PI	RCA1-3 - mPFC
CAR		-0.939, p>0.1	-0.137, p>0.1		-0.202, p>0.1	-0.217, p>0.1
Slope		0.652, p>0.1	0.385, p>0.1		1.385, p>0.1	0.660, p>0.1
AUC _g		-0.625, p>0.1	-0.484, p>0.1		-0.411, p>0.1	0.003, p>0.1

Supplementary File 1t. Correlating change in subfield functional network and diurnal cortisol indices in *Affect*. Main focus was on CA1-3 based on volumetric observations and are highlighted in bold. For these multiple comparisons (FDRq, corrected for two subfields) values are reported if uncorrected p values are below p<.05.

	LSUB	LCA1-3	LCA4/DG	RSUB	RCA1-3	RCA4/DG
CAR	0,066, p>0.1	-0,476, p>0.1	-0,535, p>0.1	-0,764, p>0.1	-0,425, p>0.1	-0,534, p>0.1
Slope	0,800, p>0.1	2,653, p=0.009, q=0.018	1,662, p>0.1	1,385, p>0.1	0,773, p>0.1	1,102, p>0.1
AUC _g	0,914, p>0.1	2,261, p=0.026, q=0.052	1,638, p>0.1	-0,697, p>0.1	0,024, p>0.1	-0,447, p>0.1

Supplementary File 1u. Correlating change in subfield volume and diurnal cortisol indices in *Presence*. Main focus was on CA1-3 based on volumetric observations and are highlighted in bold. For these multiple comparisons (FDRq, corrected for two subfields) values are reported if uncorrected p values are below p<.05.

	LSUB	LCA1-3	LCA4/DG	RSUB	RCA1-3	RCA4/DG
CAR	1,520, p>0.1	1,484, p>0.1	1,908, p=0.06	1,391, p>0.1	0,241, p>0.1	0,891, p>0.1
Slope	-1,052, p>0.1	-1,777, p=0.08	-2,890, p=0.005, q=0.03	-1,408, p>0.1	-1,736, p=0.086	-0,451, p>0.1
AUC _g	0,023, p>0.1	-0,356, p>0.1	-0,546, p>0.1	-0,223, p>0.1	-0,118, p>0.1	1,116, p>0.1

Supplementary File 1v. Correlating change in subfield volume and diurnal cortisol indices in *Perspective*. Main focus was on CA1-3 based on volumetric observations and are highlighted in bold. For these multiple comparisons (FDRq, corrected for two subfields) values are reported if uncorrected p values are below p<.05.

	LSUB	LCA1-3	LCA4/DG	RSUB	RCA1-3	RCA4/DG
CAR	0,394, p>0.1	0,575, p>0.1	0,916, p>0.1	1,535, p>0.1	0,868, p>0.1	2,263, p=0.026, q>0.1
Slope	-0,388, p>0.1	-1,409, p>0.1	-0,904, p>0.1	0,825, p>0.1	-1,152, p>0.1	-0,690, p>0.1
AUC _g	-1,136, p>0.1	-1,060, p>0.1	-0,018, p>0.1	0,995, p>0.1	-0,838, p>0.1	-0,727, p>0.1

Supplementary File 1w. Correlating change in subfield function and diurnal cortisol indices in *Presence*. Main focus was on CA1-3 based on volumetric observations and are highlighted in bold. For these multiple comparisons (FDRq, corrected for two subfields) values are reported if uncorrected p values are below p<.05.

	LSUB	LCA1-3	LCA4/DG	RSUB	RCA1-3	RCA4/DG
CAR	1,250, p>0.1	1,133, p>0.1	0,608, p>0.1	0,877, p>0.1	-0,090, p>0.1	0,160, p>0.1
Slope	0,637, p>0.1	1,103, p>0.1	1,187, p>0.1	0,096, p>0.1	0,424, p>0.1	-0,243, p>0.1
AUC _g	0,514, p>0.1	1,102, p>0.1	0,546, p>0.1	-0,130, p>0.1	-0,644, p>0.1	-1,007, p>0.1

Supplementary File 1x. Correlating change in subfield function and diurnal cortisol indices in *Presence*, *Affect*, and *Perspective*. Main focus was on CA1-3 based on volumetric observations and are highlighted in bold. For these multiple comparisons (FDRq, corrected for two subfields) values are reported if uncorrected p values are below p<.05.

	LSUB	LCA1-3	LCA4/DG	RSUB	RCA1-3	RCA4/DG
CAR	-2,324, p=0.023, q>0.1	-1,685, p=0.096	-2,215, p=0.03, q>0.1	-1,464, p>0.1	0,492, p>0.1	-1,148, p>0.1
Slope	0,318, p>0.1	1,027, p>0.1	-0,096, p>0.1	1,912, p=0.06	0,556, p>0.1	1,283, p>0.1
AUC _g	-1,990, p=0.05	-0,609, p>0.1	-1,678, p>0.1	-0,748, p>0.1	-0,473, p>0.1	-0,135, p>0.1

Supplementary File 1y. Overall effects of cortisol markers on hippocampal volume in *Presence*, *Affect*, and *Perspective*. Main focus was on CA1-3 based on volumetric observations and are highlighted in bold. For these multiple comparisons (FDRq, corrected for two subfields) values are reported if uncorrected p values are below p<.05.

	LSUB	LCA1-3	LCA4/DG	RSUB	RCA1-3	RCA4/DG
CAR	1,260, p>0.1	0,491, p>0.1	1,882, p<0.1	0,487, p>0.1	-1,116, p>0.1	1,383, p>0.1
Slope	-0,561, p>0.1	-1,861, p<0.1	-1,836, p<0.1	0,667, p>0.1	-1,788, p<0.1	-1,361, p>0.1
AUC _g	-1,383, p>0.1	-2,008, p<0.05 q>0.1	-0,117, p>0.1	0,113, p>0.1	-2,117, p<0.03, q=0.06	-0,872, p>0.1

Supplementary File 1z. Overall effects of cortisol markers on hippocampal function in *Presence*, *Affect*, and *Perspective*. Main focus was on CA1-3 based on volumetric observations and are highlighted in bold. For these multiple comparisons (FDRq, corrected for two subfields) values are reported if uncorrected p values are below p<.05.

	LSUB	LCA1-3	LCA4/DG	RSUB	RCA1-3	RCA4/DG
CAR	-0,666, p>0.1	-0,896, p>0.1	-1,221, p>0.1	-1,173, p>0.1	-0,290, p>0.1	-1,131, p>0.1
Slope	1,416, p>0.1	3,024, p<0.001, q=0.002	1,949, p<0.1	1,984, p<0.05 q>0.1	0,991, p>0.1	1,284, p>0.1
AUC _g	-0,232, p>0.1	1,614, p>0.1	0,405, p>0.1	-0,919, p>0.1	-0,463, p>0.1	-0,787, p>0.1

Supplementary File 1za. Effects of hair cortisol markers on hippocampal subfield volume in *Presence*, *Affect*, and *Perspective*. Main focus was on CA1-3 based on volumetric observations and are highlighted in bold. For these multiple comparisons (FDRq, corrected for two subfields) values are reported if uncorrected p values are below p<.05. (Cortisol (HC) and Cortisone (HE)).

	LSUB	LCA1-3	LCA4/DG	RSUB	RCA1-3	RCA4/DG
HC	-0,595, p>0.1	-2,574, p=0.011, q=0.022	-0,750, p>0.1	-1,251, p>0.1	-0,199, p>0.1	-3,138, p=0.002 q=0.012
HE	-0,127, p>0.1	-0,040, p>0.1	-0,204, p>0.1	-1,765, p<0.1	-0,589, p>0.1	-0,311, p>0.1

Supplementary File 1zb. Effects of hair cortisol markers on hippocampal subfield function in *Presence*, *Affect*, and *Perspective*. Main focus was on CA1-3 based on volumetric observations and are highlighted in bold. For these multiple comparisons (FDRq, corrected for two subfields) values are reported if uncorrected p values are below p<.05. (Cortisol (HC) and Cortisone (HE)). (Cortisol (HC) and cortisone (HE)).<colcnt=2>

	LSUB	LCA1-3	LCA4/DG	RSUB	RCA1-3	RCA4/DG
HC	-2,890, p=0.005, q=0.03	-2,700, p=0.008 q=0.013	-1,675, p>0.1	-0,638, p>0.1	-0,019, p>0.1	-0,329, p>0.1
HE	-0,627, p>0.1	0,237, p>0.1	-0,791, p>0.1	0,983, p<0.1	0,916, p>0.1	0,798, p>0.1

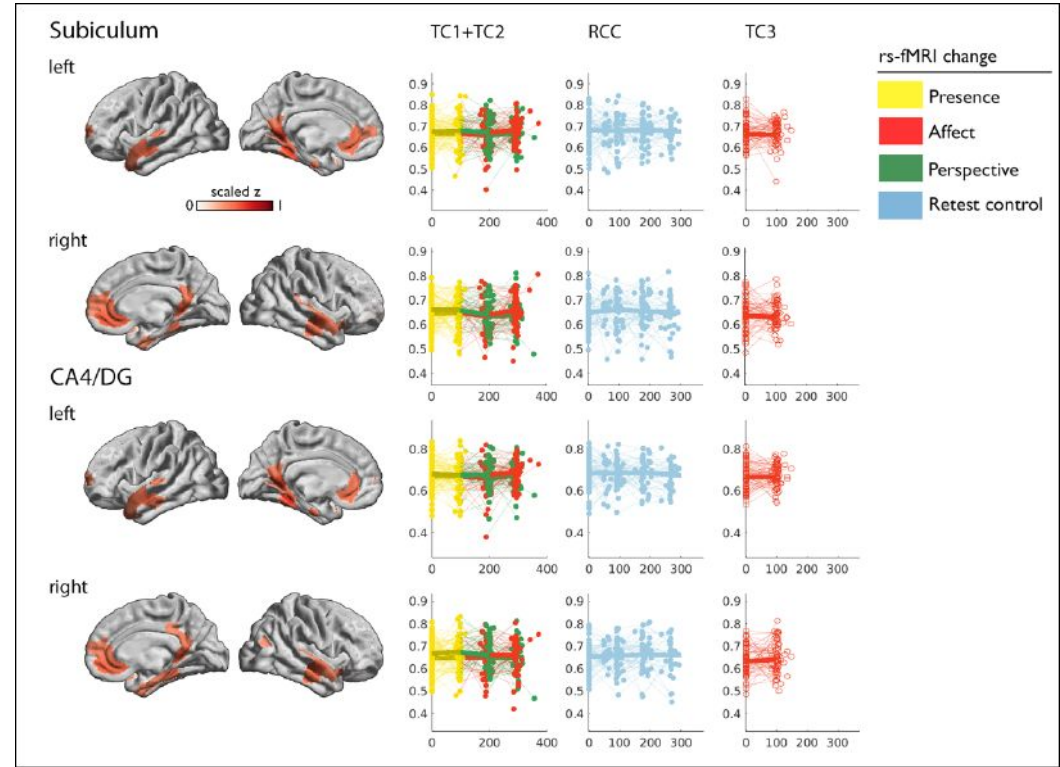


Figure 2 – Figure supplement 1.

Mean change in functional network of subiculum and CA4/DG.

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Reviewer #1 (Public Review):

Valk and Engert et al. examined the potential relations between three different mental training modules, hippocampal structure and functional connectivity, and cortisol levels (stress) over a 9-month period. They found that among the three types of mental training: Presence (attention and introspective awareness), Affect (socio-emotional - compassion and prosocial motivation), and Perspective (socio-cognitive - metacognition and perspective taking) modules; Affect training most robustly related to changes in hippocampal structure and function - specifically, CA1-3 subfields of the hippocampus. Moreover, change in intrinsic

functional connectivity related to changes in diurnal cortisol release and long-term cortisol exposure. These changes are proposed to result from a combination of factors, which is supported by multivariate analyses showing changes across subfields and training content relate to cortisol changes.

The authors demonstrate that mindfulness training programs are a potential avenue for stress interventions that impact hippocampal structure and cortisol, providing a promising approach to improve health. The data contribute to the literature on plasticity of hippocampal subfields during adulthood, the impact of mental training interventions on the brain, and the link between CA1-3 and both short- and long-term stress changes.

The authors thoughtfully approached the study of hippocampal subfields, utilizing a method designed for T1w images that outperformed Freesurfer 5.3 and that produced comparable results to an earlier version of ASHS. The authors note the limitations of their approaches and provide detailed information on the data used and analyses conducted. The results provide a strong basis from which future studies can expand using computational approaches or more fine-grained investigations of the impact of mindfulness training on cortisol levels and the hippocampus.

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Author response:

The following is the authors' response to the original reviews.

Public Reviews:

Reviewer #1 (Public Review):

Valk and Engert et al. examined the potential relations between three different mental training modules, hippocampal structure and functional connectivity, and cortisol levels over a 9-month period. They found that among the three types of mental training: Presence (attention and introspective awareness), Affect (socio-emotional - compassion and prosocial motivation), and Perspective (socio-cognitive - metacognition and perspective taking) modules; Affect training most consistently related to changes in hippocampal structure and function - specifically, CA1-3 subfields of the hippocampus. Moreover, decreases in diurnal cortisol correlated to bilateral increases in volume, and decreases in diurnal and chronic cortisol left CA1-3 functional connectivity. Chronic cortisol levels also related to right CA4/DG volume and left subiculum function. The authors demonstrate that mindfulness training programs impact hippocampus and are a potential avenue for stress interventions, a potential avenue to improve health. The data contribute to the literature on plasticity of hippocampal subfields during adulthood, the impact of mental training interventions on the brain, and the link between CA1-3 and both short- and long-term stress changes. Additional clarification and extension of the methods is needed to strengthen the authors' conclusions.

We thank the Reviewer for their positive evaluation and summary of our findings and work. We made additional changes as suggested by the Reviewer and hope this clarified any open points.

(1) The authors thoughtfully approached the study of hippocampal subfields, utilizing a method designed for T1w images that outperformed Freesurfer 5.3 and that produced comparable results to an earlier version of ASHS. However, given the use of normalized T1-weighted images to delineate hippocampal subfield volume, some caution may be warranted (Wisse et al. 2020). While the authors note the assessment of quality control

processes, the difficulty in ensuring valid measurement is an ongoing conversation in the literature. This also extends to the impact of functional co-registration using segmentations. I appreciate the inclusion of Table 5 in documenting reasons for missing data across subjects. Providing additional details on the distribution of quality ratings across subfields would help contextualize the results and ensure there is equal quality of segmentations across subfields.

We thank the Reviewer for bringing up this point. In the current work, we assessed the overall segmentation of all six subfields per individual. Thus, unfortunately, we have no data of quality of segmentation of individual subfields beyond our holistic assessment. Indeed, registration of hippocampal subfields remains a challenge and we have further highlighted this limitation in the Discussion of the current work.

“It is of note that the current work relies on a segmentation approach of hippocampal subfields including projection to MNI template space, an implicit correction for total brain volume through the use of a stereotaxic reference frame. Some caution for this method may be warranted, as complex hippocampal anatomy can in some cases lead to over- as well as underestimation of subfield volumes, as well as subfield boundaries may not always be clearly demarcated (1). Future work, studying the hippocampal surface at higher granularity, for example through unfolding the hippocampal sheet (2-5), may further help with both alignment and identification of not only subfield-specific change but also alterations as a function of the hippocampal long axis, a key dimension of hippocampal structural and functional variation that was not assessed in the current work (6, 7).”

(2) Given the consistent pattern of finding results with CA1-3, in contrast to other subfields, it would help to know if the effects of the different training modules on subfields differed from each other statistically (i.e., not just that one is significant, and one is not) to provide an additional context of the strength of results focused on Affect training and CA1-3 (for example, those shown in Figure 3).

Our work investigated i) whether the effects of the individual Training Modules differed from each other statistically. We found that the Affect Training Module showed increases in CA1-3 volume, and that these increases remained when testing effects relative to changes in this subfield following Perspective training and in retest controls. Moreover, in CA1-3 we found changes in functional connectivity when comparing the Affect to Perspective training Module. These changes were only present in this contrast, but not significant in each of the Training Modules per se. To test for specificity, we additionally evaluated whether subfield-specific changes were present above and beyond changes in the other ipsilateral hippocampal subfields. Relative to other subfields, right CA1-3 showed increases in the Affect vs Perspective contrast (left: t-value: 2.298, $p=0.022$, $Q>0.1$; right: t-value: 3.045, $p=0.0025$, $Q=0.015$). No other subfield showed significant changes. We now include this statement in the revised Results and Supplementary Tables.

“Moreover, associations between CA1-3 and Affect, relative to Perspective, seemed to go largely above and beyond changes in the other subfields (left: t-value: 2.298, $p=0.022$, $Q>0.1$; right: t-value: 3.045, $p=0.0025$, $Q=0.015$, see further Supplementary File 1h).”

Author response table 1.

Subfield-specific changes following the Training Modules, controlling for the other two ipsilateral subfields

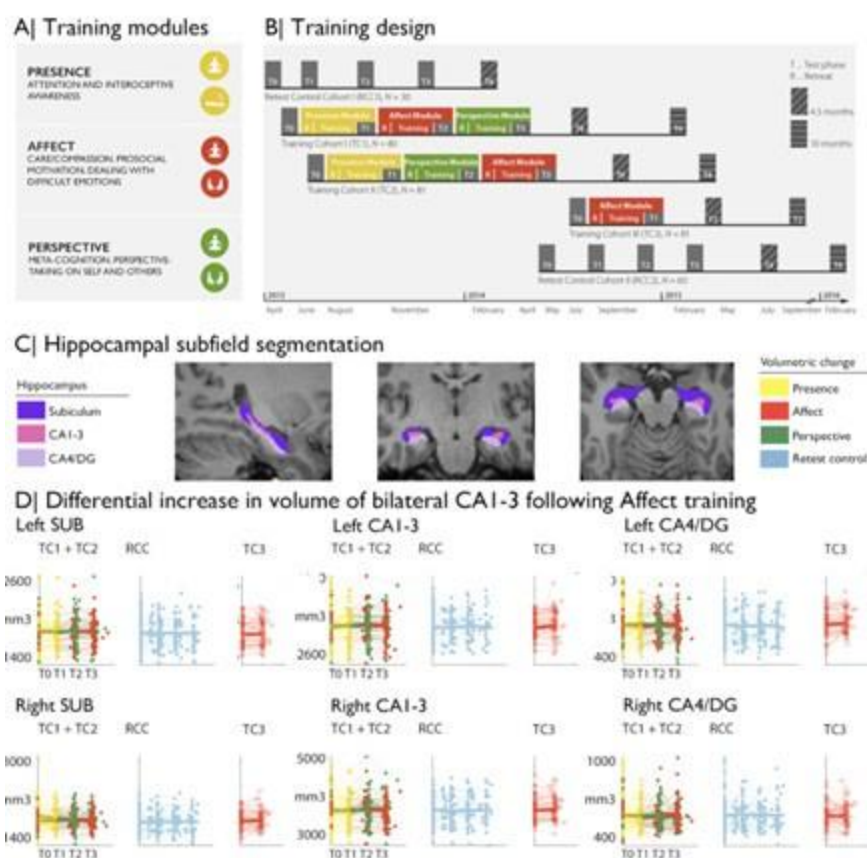
	LSUB	LCA1-3	LCA4/DG	RSUB	RCA1-3	RCA4/DG
<i>Presence vs Active</i>	$t=0.475$ $p>0.1$	$t=-0.033$ $p>0.1$	$t=-1.514$ $p>0.1$	$t=-1.365$ $p>0.1$	$t=-0.162$ $p>0.1$	$t=-0.210$ $p>0.1$
<i>Control</i>	$D=0.069$	$D=-0.004$	$D=-0.216$	$D=-0.195$	$D=-0.023$	$D=-0.03$
<i>Affect vs Perspective</i>	$t=0.793$ $p>0.1$	$t=2.298$ $p=0.022$	$t=0.924$ $p>0.1$	$t=0.883$ $p>0.1$	$t=3.045$ $p=0.0025$	$t=-0.978$ $P>0.1$
	$D=-0.03$	$D=0.275$	$D=-0.012$	$D=-0.018$	$Q=0.015$ $D=0.364$	$D=-0.117$

Reviewer #1 (Recommendations For The Authors):

(1) In Figure 1, using different colors for subfields versus the modules (yellow, red, green) would help as it could lead the reader to try to draw connections between the two when it is namely a depiction of the delineations.

As suggested, we updated Figure 1 accordingly and present the subfields in different shades of purple for clarity. Please find the updated figure below.

Author response image 1.



(2) In the Results, it was at times hard to follow when Affect off Perspective where the focus of the results. Perhaps the authors could restructure or add additional context for clarity.

We are happy to clarify. For the first analysis on Module-specific changes in hippocampal subfield volume, we compared effects across Training Modules. Here, main contrasts were

ran between subjects: Presence vs active control and within subjects: Affect versus Perspective. In additional secondary contrasts, we studied training effects vs retest control. After observing consistent increases in bilateral CA1-3 following Affect, in the following analysis, we evaluated 1) intrinsic functional networks in main and supplementary contrasts and 2) diurnal cortisol measures within the Training modules only and all three Training Modules combined, and also adopted 3) a multivariate approach (PLS) (see comments Reviewer 2). We now also report effects of cortisol change on structural and functional subfield change in Presence and Perspective, for additional completeness and clarity.

“To study whether there was any training module-specific change in hippocampal subfield volumes following mental training, we compared training effects between all three Training Modules (Presence, Affect, and Perspective). Main contrasts were: Presence vs Active control (between subjects) and Affect vs Perspective (within subjects). Supplementary comparisons were made vs retest controls and within training groups.”

“Overall, for all hippocampal subfields, findings associated with volume increases in CA1-3 following the Affect training were most consistent across timepoints and contrasts (Supplementary File 1a-f).”

“Subsequently, we studied whether hippocampal CA1-3 would show corresponding changes in intrinsic function following the Affect mental training.”

“In particular, the moderately consistent CA1-3 volume increases following Affect training were complemented with differential functional connectivity alterations of this subfield when comparing Affect to Perspective training”

“Last, we probed whether group-level changes in hippocampal subfield CA1-3 volume would correlate with individual-level changes in diurnal cortisol indices (Presence: $n=86$; Affect: $n=92$; Perspective: $n=81$), given that the hippocampal formation is a nexus of the HPA-axis (8). We took a two-step approach. First, we studied associations between cortisol and subfield change, particularly focusing on the Affect module and CA1-3 volume based on increases in CA1-3 volume identified in our group-level analysis.”

“We observed that increases in bilateral CA1-3 following Affect showed a negative association with change in total diurnal cortisol output [...]”

“We did not observe alterations in CA1-3 volume in relation to change in cortisol markers in Presence or Perspective. Yet, for Presence, we observed association between slope and LCA4/DG change ($t=-2.89$, $p=0.005$, $q=0.03$), (Supplementary File 1uv).”

“In case of intrinsic function, we also did not observe alterations in CA1-3 in relation to change in cortisol markers in Presence or Perspective, nor in other subfields (Supplementary File 1wx).”

Author response table 2.

Correlating change in subfield volume and diurnal cortisol indices in Presence. Main focus was on CA1-3 based on volumetric observations and are highlighted in bold.

	LSUB	LCA1-3	LCA4/DG	RSUB	RCA1-3	RCA4/DG
CAR	1,520, p>0.1	1,484, p>0.1	1,908, p=0.06	1,391, p>0.1	0,241, p>0.1	0,891, p>0.1
Slope	-1,052 p>0.1	-1,777, p=0.08	-2,890, p=0.005, q=0.03	-1,408, p>0.1	-1,736, p=0.086	-0,451, p>0.1
AUC_g	0,023 p>0.1	-0,356, p>0.1	-0,546, p>0.1	-0,223, p>0.1	-0,118, p>0.1	1,116, p>0.1

Author response table 3.

Correlating change in subfield volume and diurnal cortisol indices in Perspective. Main focus was on CA1-3 based on volumetric observations and are highlighted in bold.

	LSUB	LCA1-3	LCA4/DG	RSUB	RCA1-3	RCA4/DG
CAR	0,394, p>0.1	0,575, p>0.1	0,916, p>0.1	1,535, p>0.1	0,868, p>0.1	2,263, p=0.026, q>0.1
Slope	-0,388, p>0.1	-1,409, p>0.1	-0,904, p>0.1	0,825, p>0.1	-1,152, p>0.1	-0,690, p>0.1
AUC_g	-1,136, p>0.1	-1,060, p>0.1	-0,018, p>0.1	0,995, p>0.1	-0,838, p>0.1	-0,727, p>0.1

Author response table 4.

Association between stress-markers and within functional network sub-regions in Affect and Perspective.

	<i>Affect</i>	LCA1-3 - PI	RCA1-3 - mPFC	<i>Perspective</i>	LCA1-3 - PI	RCA1-3 - mPFC
CAR		-0.939, p>0.1	-0.137, p>0.1		-0.202, p>0.1	-0.217, p>0.1
Slope		0.652, p>0.1	0.385, p>0.1		1.385, p>0.1	0.660, p>0.1
AUC_g		-0.625, p>0.1	-0.484, p>0.1		-0.411, p>0.1	0.003, p>0.1

Author response table 5.

Correlating change in subfield function and diurnal cortisol indices in Presence. Main focus was on CA1-3 based on volumetric observations and are highlighted in bold. For these multiple comparisons (FDR_q, corrected for two subfields) values are reported if uncorrected p values are below p<.05.

	LSUB	LCA1-3	LCA4/DG	RSUB	RCA1-3	RCA4/DG
CAR	1,250, p>0.1	1,133, p>0.1	0,608, p>0.1	0,877, p>0.1	-0,090, p>0.1	0,160, p>0.1
Slope	0,637, p>0.1	1,103, p>0.1	1,187, p>0.1	0,096, p>0.1	0,424, p>0.1	-0,243, p>0.1
AUC_g	0,514, p>0.1	1,102, p>0.1	0,546, p>0.1	-0,130, p>0.1	-0,644, p>0.1	-1,007, p>0.1

Author response table 6.

Correlating change in subfield function and diurnal cortisol indices in Perspective. Main focus was on CA1-3 based on volumetric observations and are highlighted in bold. For these multiple comparisons (FDRq, corrected for two subfields) values are reported if uncorrected p values are below $p < .05$.

	LSUB	LCA1-3	LCA4/DG	RSUB	RCA1-3	RCA4/DG
CAR	-2,324, $p=0.023$, $q>0.1$	-1,685 , $p=0.096$	-2,215, $p=0.03$, $q>0.1$	-1,464, $p>0.1$	0,492 , $p>0.1$	-1,148, $p>0.1$
Slope	0,318, $p>0.1$	1,027 , $p>0.1$	-0,096, $p>0.1$	1,912, $p=0.06$	0,556 , $p>0.1$	1,283, $p>0.1$
AUC_g	-1,990, $p=0.05$	-0,609 , $p>0.1$	-1,678, $p>0.1$	-0,748, $p>0.1$	-0,473 , $p>0.1$	-0,135, $p>0.1$

(3) In the Methods, the authors note that corrections for multiple comparisons were used where needed, throughout the manuscript there is some switching between corrected and uncorrected p-values. At times, this made it difficult to follow in terms of when these corrections were needed.

For clarity, we added explicit multiple comparisons information a) in main and supplementary results, and b) wherever extra information was needed. Also, we only included main contrasts in Table 1-3 to avoid confusion and moved the information on changes in SUB and CA4/DG to the Supplementary tables.

(4) Typically, when correcting for intracranial volume the purpose is the ensure that sexual dimorphism in the size of the brain is accounted for. I would recommend the authors assess whether sex differences are accounted for by the MNI normalization approach taken. In the reading of the original Methods paper for the patch-based algorithm used, ICV was used to transform to MNI152 space. It would help to have additional information on how the normalization was done in the current study in order to draw comparisons to other findings in the literature.

We are happy to further clarify. In the current work, we used the same approach as in the original paper. Volumes were linearly registered to the MNI template using FSL flirt. We now provided this additional information in the revised methods.

“Hippocampal volumes were estimated based on T1w data that were linearly registered to MNI152 using FSL flirt (<http://www.fmrib.ox.ac.uk/fsl/>), such that intracranial volume was implicitly controlled for.”

We agree with the Reviewer that sex differences may still be present, and investigated this. At baseline, sex differences were found in all subfields in the left hemisphere, and right CA4/DG ($FDRq < 0.05$). Regressing out ICV resolved remaining sex differences. We then evaluated whether main results of volumetric subfield change were impacted by ICV differences. Differences between Affect and Perspective remained stable. We have now added this additional analysis in the Supplementary Materials.

“Although stereotaxic normalization to MNI space would in theory account for global sex differences in intra-cranial volume, we still observed sex differences in various subfield volumes at baseline. Yet, accounting for ICV did not impact our main results suggesting

changes in CA1-3 following Affect were robust to sex differences in overall brain volume (Supplementary File1j).”

Author response table 7.

Sex differences (female versus male) in hippocampal subfield volumes.

	LSUB	LCA1-3	LCA4/DG	RSUB	RCA1-3	RCA4/DG
baseline	t=2.423 p=0.008 q=0.049	t=2.061 p=0.021 q=0.121	t=3.671 p<0.001 q=0.001	t=-0.520 p=0.698 q>0.05	t=3.570 p<0.001 q>0.002	t=2.555 p=0.006 q=0.034
baseline+ ICVcontrol	t=1.739 p=0.042 q>0.1	t=-0.171 p>0.1 q>0.1	t=1.259 p>0.1 q>0.1	t=-0.358 p>0.1 q>0.1	t=1.962 p=0.025 q>0.1	t=1.201 p>0.1 q>0.1
Main change analysis						
Affect versus Perspective ICVcontrol	t=0.514 p>0.05 q>0.05	t=2.588 p=0.01 q=0.06	t=0.677 p>0.05 q>0.05	t=-0.262 p>0.05 q>0.05	t=3.065 p=0.002 q=0.014	t=-0.511 p>0.05 q>0.05
Presence versus Active Control ICVcontrol	t=-0.101 p>0.05 q>0.05	t=0.214 p>0.05 q>0.05	t=1.236 p>0.05 q>0.05	t=1.462 p>0.05 q>0.05	t=0.256 p>0.05 q>0.05	t=0.406 p>0.05 q>0.05

Reviewer #2 (Public Review):

In this study, Valk, Engert et al. investigated effects of stress-reducing behavioral intervention on hippocampal structure and function across different conditions of mental training and in relation to diurnal and chronic cortisol levels. The authors provide convincing multimodal evidence of a link between hippocampal integrity and stress regulation, showing changes in both volume and intrinsic functional connectivity, as measured by resting-state fMRI, in hippocampal subfield CA1-3 after socio-affective training as compared to training in a socio-cognitive module. In particular, increased CA1-3 volume following socio-affective training overlapped with increased functional connectivity to medial prefrontal cortex, and reductions in cortisol. The conclusions of this paper are well supported by the data, although some aspects of the data analysis would benefit from being clarified and extended.

A main strength of the study is the rigorous design of the behavioral intervention, including test-retest cohorts, an active control group, and a previously established training paradigm, contributing to an overall high quality of included data. Similarly, systematic quality checking of hippocampal subfield segmentations contributes to a reliable foundation for structural and functional investigations.

We thank the Reviewer for the thoughtful summary and appreciation of our work, as well as requests for further clarification and analyses. We addressed each of them in a point by point fashion below.

Another strength of the study is the multimodal data, including both structural and functional markers of hippocampal integrity as well as both diurnal and chronic estimates of cortisol levels.

(1) However, the included analyses are not optimally suited for elucidating multivariate interrelationships between these measures. Instead, effects of training on structure and function, and their links to cortisol, are largely characterized separately from each other. This results in the overall interpretation of results, and conclusions, being dependent on a large number of separate associations. Adopting multivariate approaches would better

target the question of whether there is cortisol-related structural and functional plasticity in the hippocampus after mental training aimed at reducing stress.

We thank the Reviewer for this suggestion. Indeed, our project combined different univariate analyses to uncover the association between hippocampal subfield structure, function, and cortisol markers. While systematic, a downside of this approach is indeed that interpretation of our results depend on a large number of analyses. To further explore the question whether there is cortisol-related structural and functional plasticity in the hippocampus, we followed the Reviewer's suggestion and additionally adopted a multivariate partial least squares (PLS) model. We ran two complementary models. One focusing on the bilateral CA1-3, as this region showed increases in volume following Affect training and differential change between Affect and Perspective training in our resting state analyses and one model including all subfields. Both models included all stress markers. We found that both models could significantly relate stress markers to brain measures, and that in particular Affect showed strong associations with significant the latent markers. Both analyses showed inverse effects of structure and function in relation to stress markers and both slope and AUC changes showed strongest loadings. We now include these analyses the revised manuscript.

Abstract

“Of note, using a multivariate approach we found that other subfields, showing no group-level changes, also contributed to alterations in cortisol levels, suggesting circuit-level alterations within the hippocampal formation.”

Methods

“Partial least squares analysis

To assess potential relationships between cortisol change and hippocampal subfield volume and functional change, we performed a partial least squares analysis (PLS) (9, 10). PLS is a multivariate associative model that to optimizes the covariance between two matrices, by generating latent components (LCs), which are optimal linear combinations of the original matrices (9, 10). In our study, we utilized PLS to analyze the relationships between change in volume and intrinsic function of hippocampal subfields and diurnal cortisol measures. Here we included all Training Modules and regressed out effects of age, sex, and random effects of subject on the brain measures before conducting the PLS analysis. The PLS process involves data normalization within training groups, cross-covariance, and singular value decomposition. Subsequently, subfield and behavioral scores are computed, and permutation testing (1000 iterations) is conducted to evaluate the significance of each latent factor solution (FDR corrected). We report then the correlation of the individual hippocampal and cortisol markers with the latent factors. To estimate confidence intervals for these correlations, we applied a bootstrapping procedure that generated 100 samples with replacement from subjects' RSFC and behavioral data.”

Results

“Last, to further explore the question whether there is concordant cortisol-related structural and functional plasticity in the hippocampus we adopted a multivariate partial least square approach, with 1000 permutations to account for stability (9, 10) and bootstrapping (100 times) with replacement. We ran two complementary models including all Training Modules whilst regressing out age, sex and random effects of subject. First, we focused on the bilateral CA1-3, as this region showed increases in volume following Affect training and differential change between Affect and Perspective training in our resting state analyses. In the second model included structural and functional data of all subfields. Both models included all stress markers. We found that both models could identify significant associations between cortisol stress markers and hippocampal plasticity ($FDRq < 0.05$), and that in particular Affect showed

strongest associations with the latent markers for CA1-3 (Table 5). Both analyses showed inverse effects of subfield structure and function in relation to stress markers and both slope and AUC changes showed strongest associations with the latent factor.”

Author response table 8.

Multivariate PLS analyses linking cortisol markers to hippocampal subfield volume and function.

	LC1	Overall	Presence	Affect	Perspective
CA1-3	p<0.01, 67%	r=0.20	r=0.17	r=0.27	r=0.16
all	p<0.01, 71%	r=0.24	r=0.16	r=0.30	r=0.26

Discussion

“Last, performing multivariate analysis, we again observed associations between CA1-3 volume and function plasticity and stress change, strongest in Affect. Yet combining all subfields in a single model indicated that other subfields also link to stress alterations, indicating that ultimately circuit-level alterations within the hippocampal formation relate to latent changes in diurnal stress markers across Training Modules.”

“This interpretation is also supported by our multivariate observations.”

“In line with our observations in univariate analysis, we found multivariate associations between hippocampal subfield volume, intrinsic function and cortisol markers. Again, the contribution of volume and intrinsic function was inverse. This may possibly relate to the averaging procedure of the functional networks. Combined, outcomes of our univariate and multivariate analyses point to an association between change in hippocampal subfields and stress markers, and that these changes, at the level of the individual, ultimately reflect complex interactions within and across hippocampal subfields and may capture different aspects of diurnal stress. Future work may more comprehensively study the plasticity of the hippocampal structure, and link this to intrinsic functional change and cortisol to gain full insights in the specificity and system-level interplay across subfields, for example using more detailed hippocampal models (3). Incorporating further multivariate, computational, models is needed to further unpack and investigate the complex and nuanced association between hippocampal structure and function, in particular in relation to subfield plasticity and short and long-term stress markers.”

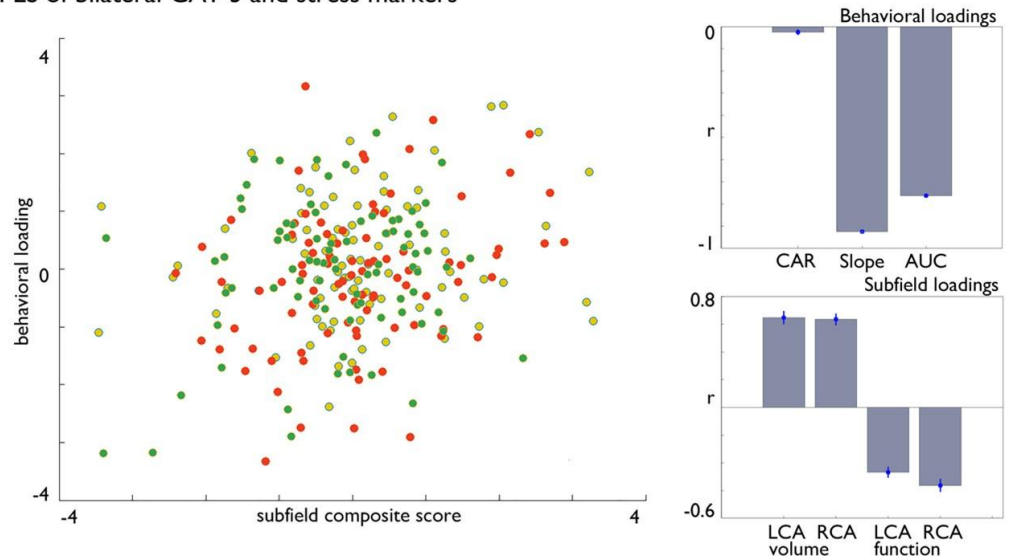
“...based on univariate analysis. Our multivariate analysis further nuanced this observation, but again pointed to an overall association between hippocampal subfield changes and cortisol changes, but this time more at a systems level.”

“Lastly, our multivariate analyses also point to a circuit level understanding of latent diurnal stress scores.”

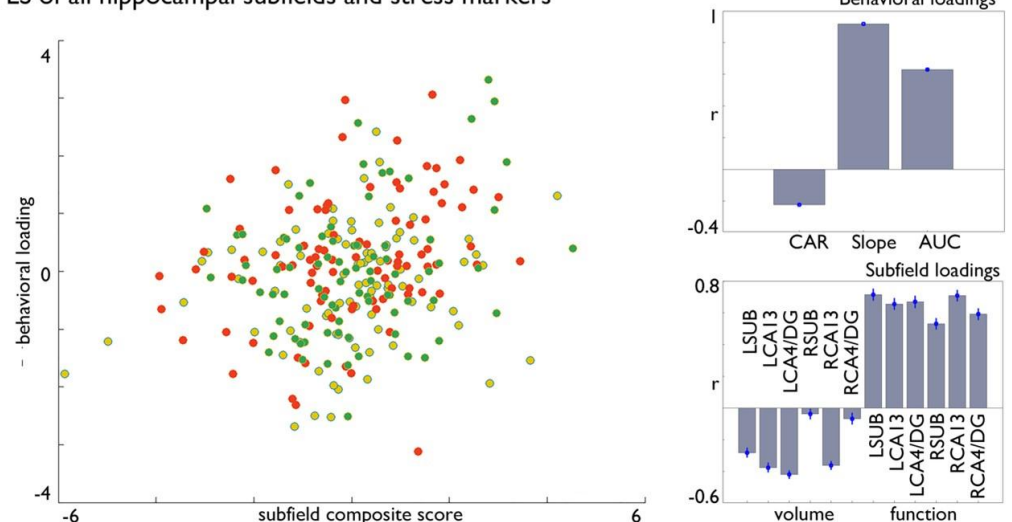
Author response image 2.

Multivariate associations between changes in structure and function of hippocampal subfield volume and markers of stress change in Affect. A) Multivariate associations between bilateral CA1-3 volume and intrinsic function and stress markers. Left: Scatter of loadings, colored by Training Module; Right upper: individual correlations of stress markers; Right lower: individual correlation of subfields; B). Multivariate associations between all subfields' volume and intrinsic function and stress markers. Left: Scatter of loadings, colored by Training Module; Right upper: individual correlations of stress markers; Right lower: individual correlation of subfields.

A) PLS of bilateral CA1-3 and stress markers



B) PLS of all hippocampal subfields and stress markers



(2) The authors emphasize a link between hippocampal subfield CA1-3 and stress regulation, and indeed, multiple lines of evidence converge to highlight a most consistent role of CA1-3. There are, however, some aspects of the results that limit the robustness of this conclusion. First, formal comparisons between subfields are incomplete, making it difficult to judge whether the CA1-3, to a greater degree than other subfields, display effects of training.

We thank the Reviewer for this comment. To further test for specificity, we additionally evaluated subfield-specific changes relative to other subfields for our main contrasts (Presence versus Active Control and Affect versus Perspective). Relative to other subfields, right CA1-3 showed increases in the Affect vs Perspective contrast (left: t-value: 2.298, $p=0.022$, $Q>0.1$; right: t-value: 3.045, $p=0.0025$, $Q=0.015$); no other subfield showed significant changes. We now include this statement in Results and Supplementary Tables.

“Moreover, associations between CA1-3 and Affect, relative to Perspective, seemed to go largely above and beyond changes in the other subfields (left: t-value: 2.298, $p=0.022$, $Q>0.1$; right: t-value: 3.045, $p=0.0025$, $Q=0.015$, see further Supplementary File 1h).”

Author response table 9.

Subfield-specific changes following the Training Modules, controlling for the other two ipsilateral subfields

	LSUB	LCA1-3	LCA4/DG	RSUB	RCA1-3	RCA4/DG
<i>Presence vs</i>	t=0.475	t=-0.033	t=-1.514	t=-1.365	t=-0.162	t=-0.210
<i>Active</i>	p>0.1	p>0.1	p>0.1	p>0.1	p>0.1	p>0.1
<i>Control</i>	D=0.069	D=-0.004	D=-0.216	D=-0.195	D=-0.023	D=-0.03
<i>Affect vs</i>	t=0.793	t=2.298,	t=0.924	t=0.883	t=3.045	t=-0.978
<i>Perspective</i>	p>0.1	p=0.022	p>0.1	p>0.1	p=0.0025	p>0.1
	D=-0.03	D=0.275	D=-0.012	D=-0.018	q=0.015 D=0.364	D=-0.117

(3) Relatedly, it would be of interest to assess whether changes in CA1-3 make a significant contribution to explaining the link between hippocampal integrity and cortisol, as compared to structure and functional connectivity of the whole hippocampus.

We thank the Reviewer for this comment. Please see the PLS analysis performed above (R2Q1). Indeed, not only CA1-3 but also other subfields seem to show a relationship with cortisol, in line with circuit level accounts on stress regulation and hippocampal circuit alterations (8, 11-15).

(4) Second, both structural and functional effects (although functional to a greater degree), were most pronounced in the specific comparison of "Affect" and "Perspective" training conditions, possibly limiting the study's ability to inform general principles of hippocampal stress-regulation.

We agree with the Reviewer that the association between stress and hippocampal plasticity, on the one hand, and mental training and hippocampal plasticity, on the other hand, make it not very straightforward to inform general principles on hippocampal stress regulation. However, as underscored in the discussion, in previous work we could also link mental training to stress reductions(16-18). We hope that the additional analyses and explanations further explain the multilevel insights of the current work, on the one hand using group-level analysis to investigate and illustrate the association between mental training and hippocampal subfield volume and intrinsic function, and on the other hand using individual level analysis to unpack the association between cortisol change and hippocampal subfield change.

Reviewer #2 (Recommendations For The Authors):

(1) In the Results, the description of how the hippocampal subfields' functional networks were defined would benefit from some clarification. It is also somewhat unclear what is meant by (on page 10): "Evaluating functional connectivity changes, we found that connectivity of the right CA1-3 functional network showed differential changes when comparing Affect training to Perspective training (2.420, $p=0.016$, $FDRq=0.032$, Cohens $D=0.289$), but not versus retest control (Table 1 and Supplementary Table 8-14)." Were there significant changes in CA1-3 FC following both training conditions (but these differed from each other)? A description of what this difference reflected would increase the reader's understanding.

We are happy to clarify. We included information of change of individual modules in the Supplementary materials, Supplementary Table 1 and 2, 9 and 10. Changes for functional connectivity were largely due to the differences in Modules, but did not show strong effects in

one Module alone. We now include information on Affect and Perspective un-contrasted change in the main results text:

“... which could be attributed to decreases in right CA1-3 mean FC following Perspective ($t=-2.012$, $p=0.045$, $M=-0.024$, $std: 0.081$, $CI [-0.041 -0.006]$), but not Affect ($t=1.691$, $p=0.092$, $M: 0.010$, $std: 0.098$, $CI [-0.01 0.031]$); changes were not present when comparing Affect training versus retest control (Table 1 and Supplementary File 1k-q).”

(2) As described in the Public Review, the lack of multivariate assessments may risk selling the data short. Including analyses of concomitant functional and structural changes, in relation to cortisol, seems like an approach better adapted to characterize meaningful interrelationships between these measures.

We thank the Reviewer for suggesting multivariate assessments. To understand the interrelation between behavioral intervention, hippocampal plasticity, and cortisol changes, the current work first evaluates a simpler operationalization of the relationship between hippocampal subfield structure and volume, and cortisol as a function of mental training. Thus, given the complex nature of the study, we initially opted for a model where we assess structural and functional changes independently, with structural changes as the basis of our investigations. Now we have also included a multivariate approach (PLS) to further test the association between hippocampal subfields and cortisol markers, please see our additions to the manuscript above. We now highlighted multivariate associations in the Discussion as well, and suggest this as an important next step for more detailed, future investigations.

“Incorporating further multivariate, computational, models is needed to further unpack and investigate the complex and nuanced association between hippocampal structure and function, in particular in relation to subfield plasticity and short and long-term stress markers.”

(3) A minor comment regards the Figures. Some main effects should be visualized in a clearer manner. For instance, the scatterplots in Figure 1, panel D. Also, some of the current headings within the figures could be made more intuitive to the reader.

We thank the Reviewer for this comment. To improve clarity, we updated figure headings. For Figure 1D, the challenge is that the data are quite scattered and we aimed to visualize our observations in a naturalistic way. Therefore, we added additional y-axis information to further clarify the figures. Creating more overlap or differentiation would make other elements of the figure less clear, hence we remained with the current set-up detailing the intra- and inter-individual alterations of the current model.

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