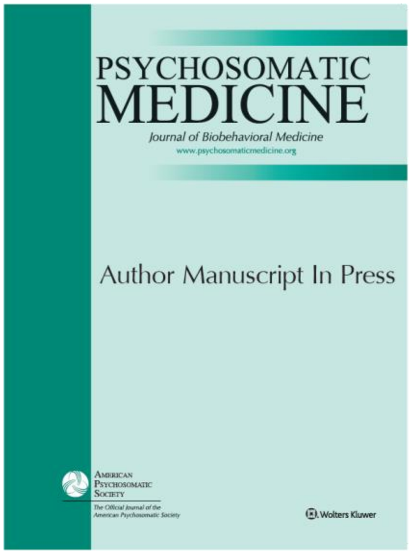

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Contemplative mental training reduces hair glucocorticoid levels in a randomized clinical trial

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Abstract

Objective: To investigate the effect of regular contemplative mental training on endocrine and psychological indices of long-term stress.

Methods: An open-label efficacy trial that comprised three distinct 3-month modules targeting attention and interoception, socio-affective or socio-cognitive abilities through dyadic exercises and secularised meditation practices was conducted with healthy adults. Participants underwent the training for three months, nine months, or were assigned to a retest control cohort. Chronic stress indices were assayed at four timepoints: pre-training and after three, six and nine months. The main outcome measures were cortisol (HC) and cortisone (HE) concentrations in hair and self-reported long-term stress.

Results: Of 362 initially randomized individuals, 30 dropped out before study initiation (N=332; mean age-40. 7 ± SD=9.2 years; 197 women). Hair-based glucocorticoid assays were available from n=227, and questionnaire data from n=326. Results from three separate training cohorts (TCs) revealed consistent decreases in HC and HE levels over the first three (TC3) to six months (TC1 and TC2) of training, with no further reduction at the final 9-month mark (baseline to end-of-training, *HC: TC1*, *t*(355)=2.59, *p*=.010; est.:0.35[0.14]; *TC2*, *t*(363)=4.06, *p*<.001; est.:0.48[0.12]; *TC3:* *t*(368)=3.18, *p*=.002; est.:0.41[0.13]; *HE: TC1***,** *t*(435)=3.23, *p*=.001; est.:0.45[0.14]; *TC2:* *t*(442)=2.60, *p*=.010; est.:0.33[0.13]; *TC3:* *t*(446)=4.18, *p*<.001; est.:0.57[0.14]). Training effects on HC increased with practice frequency, and effects on both HC and HE were independent of training content and unrelated to change in self-reported chronic stress. Self-reported stress, and cortisol to dehydroepiandrosterone ratios as an exploratory endpoint, were also reduced, albeit less consistently.

Conclusions: Our results point to the reduction of long-term cortisol exposure as a mechanism through which contemplative mental training may exert positive effects on practitioners' health.

Trial registration: ClinicalTrials.gov identifier: NCT01833104

Key Words: Contemplative mental training, hair cortisol, glucocorticoids, objective and subjective stress.

Abbreviations: HC, hair cortisol; HE, hair cortisone; TC1-3, training cohorts 1-3.

Introduction

Rising prevalence of stress-related mental and physical disorders (1,2) has led to the recognition of chronic stress as one of the 21st century's major health risks (3). The health outcomes of exposure to psychosocial stress are mediated by prolonged activation of our main neuroendocrine stress systems, the sympathetic-adrenal-medullary (SAM) and the hypothalamic-pituitary-adrenal (HPA) axes. Both systems exert complex effects on immune and metabolic processes, and are causally involved in the development of cardiovascular, metabolic, and autoimmune disorders, among others (4). In striving to reduce stress and promote health and wellbeing, secular meditation-based mental training interventions, such as the mindfulness-based stress reduction (MBSR) program (5), have gained popularity. Various health-related benefits have been associated with engagement in such training interventions (see e.g. 6,7 for meta analyses). Findings from our own 9-month mental training study, the ReSource Project (8), show differential positive changes in subjective well-being, cognition, peripheral physiology, and brain plasticity following distinct types of contemplative mental training (9).

Of particular interest for clinical application are the downstream health benefits of contemplative training, such as mitigation or prevention of stress-related disorders. Current theory suggests that these outcomes are mediated by dampened activity of physiological stress systems, above all the HPA axis (10). In line with this theory, subjective-psychological stress load is one of the most widely reported training outcomes (7). At the same time, self-report measures of contemplative training effects may be particularly vulnerable to confounds such as demand-effects and expectancy bias, since the training trials are inevitably open-label. Researchers are thus increasingly relying on physiological measures as more reliable and objective health outcomes. Results from such studies have shown that although correspondence between psychological and physiological measures of stress is often assumed, evidence for training-related endocrine stress reduction in healthy participants is currently mixed and inconclusive: Studies of mental training effects on stress-related biomarkers predominantly focus on the secretion of the HPA axis output hormone cortisol, either in response to acute stress or during basal activity, measured in blood or saliva. First evidence for reduced cortisol output after psychosocial stress induction was found immediately after a single mindfulness-based meditation session preceded by five days of practice (11). In comparing different practice types, we identified reduced cortisol secretion in response to an acute psychosocial laboratory stressor following the 3-month long training of either socio-affective or socio-cognitive practices, but not after the training of present-moment attention and interoception (12). Several other studies of psychosocial stress induction found no effects of mindfulness- or compassion-based training on acute cortisol release (e.g. 13, 14; for a review see also 15). Similarly heterogeneous results emerge at the level of basal HPA axis activity. Reports of lower diurnal cortisol output mainly stem from mindfulness-based interventions employing the MBSR program, for which reductions in the cortisol awakening response (CAR) and afternoon/evening cortisol levels have been found in healthy as well as diseased individuals (16–18). Again, these findings are contrasted by several null results (19,20).

These mixed outcomes do not sufficiently corroborate the hypothesis that reduced HPA-axis activity mediates long-term training-related health benefits. Notably, however, while acute and diurnal cortisol indices provide a window to an individual's long-term cortisol exposure, both bear shortcomings as measures of chronic stress. Cortisol levels collected after acute challenge reflect stress responses in a highly specific setting, and indices of diurnal cortisol measured in saliva, blood, or urine fluctuate considerably from day-to-day (21,22). Since it is the long-term, cumulative HPA axis activation that is particularly maladaptive and related to ill-health (4,23), methodological limitations in capturing chronic physiological stress may account for some of the heterogeneity in the contemplative training literature.

The present study aimed to investigate whether contemplative mental training affects patterns of long-term cortisol secretion as a mediator of downstream health benefits in 227 healthy adults. Instead of acute or diurnal cortisol secretion, we utilized the method of hair cortisol (HC) and cortisone (HE) assessment as indices of the long-term physiological stress load. HC and HE concentrations are assumed to capture systemic (i.e., whole body) cortisol exposure and have been linked to the experience of psychosocial stress (24). HC concentration is also positively correlated with diurnal cortisol output (12,24), but less prone to state-related variance, which may allow for a particularly stable prediction of whether mental training has a long-term impact on HPA axis activity. Alongside cortisol, it has been suggested that levels of the inactive cortisol metabolite and precursor molecule cortisone yield a complementary, potentially more stable glucocorticoid signal (25; supplement). We thus assayed cortisol and cortisone levels in 3 cm proximal hair segments, corresponding to approximately 3 months exposure. In light of slowly increasing evidence for hair glucocorticoid levels as indicators of long-term cortisol exposure upon planning of the study in 2011, HC and HE measures were registered as secondary outcomes to the clinical trial. To capture psychological stress load, self-reported chronic stress was measured using the Perceived Stress Scale (PSS; 26) and the Trier Inventory for Chronic Stress (TICS; 27).

As an exploratory endpoint, we additionally assayed dehydroepiandrosterone (DHEA) concentration in hair to assess potential training effects on the ratio of cortisol relative to DHEA expression (HC/DHEA). The anabolic functions of DHEA complement the metabolic effects of cortisol in a co-regulatory framework in which DHEA buffers the detrimental influences of cortisol signalling through neuroprotective, anti-inflammatory, antioxidative and anti-glucocorticoid effects (28–30). The ratio of cortisol to DHEA levels can be employed as an indicator for the balance between anabolic and catabolic processes (31). Although HPA axis dysregulation may be reflected in elevated levels of either hormone, high DHEA levels are generally implicated in protective and stress-resilience related processes (32–34), whereas most studies associated high cortisol/DHEA ratios with psychiatric disorders including depression, PTSD and schizophrenia (35–37) or with chronic stress in healthy adults (38). Accordingly, we explored whether patterns of change in HC/DHEA ratios may mirror HC, which would provide support for the proposition that HC reduction reflects improved regulation of HPA axis activity.

The training regimen of the ReSource Project was designed to disentangle the specific effects of three different types of mental practice. This differentiated approach is especially valuable given the multifaceted nature of many mindfulness-based programs, which typically combine diverse practice types (39). In three separate modules termed Presence, Affect and Perspective, participants trained attention-, socio-emotional or socio-cognitive based practices for 3-months each (Figure 1A). Participants were assigned either to one of two 9-month training cohorts completing all three training modules in different orders (TC1 and TC2), a 3-month Affect only training cohort (TC3) or a retest control cohort (RCC) (Figure 1B; see also 29, chapter 7). During each module, participants completed a standardized training routine involving weekly 2-hour group sessions and daily practice of core exercises.

Previous studies investigated potential effects of mindfulness-based training on HC after 7-10 weeks of group training (41–44) and 12 weeks of online interventions (45,46). Among these, only one pilot study detected significantly decreased HC in 18 participants (41). Extending on theses preliminary findings, the large-scale ReSource Project can produce conclusive results about the more longitudinal effects of a 9-month-long intervention, as well as potential differential outcomes of distinct types of contemplative practice. In light of the above outlined evidence for changes in diurnal cortisol after mindfulness-based training, we primarily expected to find decreased HC and HE levels after the attention-based Presence module, which included classic mindfulness-practices that are also central to the MBSR program. We hypothesized that training-related reduction would be observable relative to the study baseline as well as to the RCC. Because basal and stress-induced cortisol levels are not reliably associated (e.g. 35), it remained an open question whether the acute stress-reducing properties of the social Affect and Perspective modules identified in our previous study (48) would translate to reduced cortisol levels in hair. Finally, we expected decrease in self-reported long-term stress in parallel to change in physiological stress load, aligning also with consistent reports of stress-reduction after mindfulness-based training (7) and to a lesser extend after compassion-based training (49).

Materials and Methods

Participants

All participants underwent comprehensive face-to-face mental health diagnostic interviews with a trained clinical psychologist and completed additional mental health questionnaires. Volunteers were excluded if they fulfilled the criteria for an Axis-I disorder within the past two years, or for schizophrenia, psychotic disorder, bipolar disorder, substance dependency or any Axis-II disorder at any time in their life. Volunteers who had prior meditation experience or were taking medication influencing the HPA axis were also excluded (for further details on the screening procedure, see 50). The ReSource Project was registered with the Protocol Registration System of ClinicalTrial.gov (Identifier NCT01833104) and approved by the Research Ethics Boards of Leipzig University (ethic number: 376/12-ff) and Humboldt University Berlin (ethic numbers: 2013-20, 2013-29, 2014-10). The study was conducted in accordance with the Declaration of Helsinki. Participants gave written informed consent, could withdraw from the study at any time and were financially compensated.

To avoid straining participants through excessive testing in the context of the multi-measure ReSource Project, sampling of hair was presented to participants as an optional rather than a core testing procedure, leading to lower adherence rates. Of 332 initial ReSource participants (197 women; mean age ± SD: 40.74±9.24 years; age range: 20-55 years), 217 provided hair samples at baseline (T0), of which 179 could be re-assayed for the present change analysis; 157 provided samples at T1, 136 at T2 and 150 at T3 (see Figure 2 and Tables S1-S3 for sample sizes of all measures per cohort and reasons for missing cases). Twenty-four participants (18 women) were light smokers (≤10 cigarettes/day; mean ± SD: 16.01±16.09 cigarettes/week).

Training program

The ReSource Project examined the specific effects of three commonly practiced types of mental training, specifically attention-, socio-emotional or socio-cognitive based techniques. For this purpose, the training program was parceled into three separate modules (Presence, Affect, and Perspective), each of which cultivated distinct contemplative capacities over three months (Figure 1A; 40). Every module began with a 3-day retreat during which professional teachers introduced participants to the conceptual core and the relevant practices of a given module. Afterwards, participants attended weekly 2-hour group sessions, and were asked to exercise the respective module's two core practices for 30 minutes daily on five days per week using a tailor-made app and online platform.

The psychological processes targeted in the Presence module are attention and interoceptive awareness. Its core practices are Breathing Meditation and Body Scan, both of which are classical mindfulness-based exercises also implemented in the MBSR program. The Affect module targets social emotions such as compassion, loving kindness and gratitude, and aims to enhance prosocial motivation and dealing with difficult emotions. These skills are targeted through the core practices Loving-kindness Meditation, which is also featured in MBSR-type programs, and the novel Affect Dyad. Together, the Presence and Affect modules target and disentangle the two main components of the MBSR program. In the Perspective module, participants train metacognition and perspective-taking on self and others through the core practices Observing-thoughts Meditation and Perspective Dyad.

The two contemplative dyads are partner exercises that were developed for the ReSource training (51). 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 also 40). 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 (40,51).

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 52,53).

Study Design

Participants were assigned either to one of two 9-month training cohorts completing all three training modules in different orders (TC1, initial n=80, n for present study=48; and TC2, initial n=81, present n=62), a 3-month Affect only training cohort (TC3, initial n=81, present n=49) or a retest control cohort (RCC, initial n=90, present n=68) (Figure 1B; 51). Cohort assignment was completed using bootstrapping without replacement to ensure the formation of demographically homogeneous groups. TC1 and TC2 began their training with the attention-based Presence module. Subsequently, they underwent Affect and Perspective training in different orders, thus controlling for sequence effects. TC3 was conducted to isolate the specific effects of the Presence module from the Affect module. The study followed a mixed design, in which most, but not all, participants received all types of training. Training and data collection took place between April 2013 and February 2016.

Assay of steroid hormone concentration in hair

HC and HE concentrations are indicative of systemic cortisol exposure and markers of chronic stress (24). Levels of the inactive cortisol metabolite and precursor molecule cortisone have been suggested to yield a complementary, potentially more stable glucocorticoid signal alongside cortisol itself (25; supplement). While the precise mechanism behind hormone accumulation in hair is incompletely understood, it is assumed that during hair growth, free hormone molecules are continuously incorporated into follicles, proportional to their overall concentration in the physiological system. HC and HE concentrations in a 1 cm hair segment are thus assumed to indicate the cumulative systemic cortisol or cortisone exposure over an approximately 1-month period (24). The same applies to accumulation of DHEA in hair, which we assayed in an exploratory approach.

For their assessment, hair strands were taken as close as possible to the scalp from a posterior vertex position at T0 and after each training module (at T1, T2 and T3). Hair samples were wrapped in aluminum foil and stored in the dark at room temperature until assay at the Department of Psychology, TU Dresden, Germany. Based on the assumption of an average hair growth rate of 1 cm/month (Wennig, 2000), we analyzed the proximal 3 cm segment of hair to assess accumulation of cortisol, cortisone and DHEA over each 3-month period. Hormone concentrations were measured using liquid chromatography-tandem mass spectrometry (LC–MS/MS), the current gold-standard approach for hair steroid analysis (54), following our previously published protocol with a limit of quantification for cortisol and cortisone below 0.09 pg/mg and intra- and inter-assay CVs between 3.7 and 8.8% (55). All hormone concentrations were reported in pg/mg.

A first assay of samples collected at baseline was conducted in 2015, allowing researchers to address cross-sectional research questions (12) before termination of the longitudinal data collection. Thirty-eight samples were used up in this analysis. For the current longitudinal research aim, the remaining baseline samples were re-assayed jointly with all additional samples (assessed at T1, T2 and T3) to avoid potential systematic effects of storage time and minimize reagent batch effects. Specifically, all samples of one participant were always ran with the same reagent batch to avoid intra-individual variance due to batch effects.

Subjective stress measures

Self-reported chronic stress was measured on the basis of the summary score of the Perceived Stress Scale (PSS; 26), as well as the global stress score of the Trier Inventory for Chronic Stress (TICS; 27). The 10-item PSS is the most widely used psychological instrument for measuring the perception of stress. It focuses on the degree to which situations in the past month are appraised as unpredictable, uncontrollable and overloaded, and produces one summary stress score. The 39-item TICS captures a time span of 1-3 months and measures six aspects of chronic stress (work overload, worries, social stress, lack of social recognition, work discontent and intrusive memories), and one global stress score. Both questionnaires have satisfactory reliability and validity (26,27).

Measures of training engagement

To examine causes of individual variability in training effects, we assessed two measures of training engagement: practice frequency, objectively traced via our online training platform, and self-reported liking of the different training modules. Details on the measurement and analysis of both metrics are provided in the Supplementary Methods, http://links.lww.com/PSYMED/A759. Practice frequency is a particularly interesting metric as it provides insights to the impact of training dosage.

Statistical analysis

Data processing. Raw HC and HE data were each treated with a natural log transformation to remedy skewed distributions. Ratios of cortisol to DHEA (HC/DHEA) as an exploratory outcome were computed by dividing raw HC measures by raw DHEA measures, and subsequently also treated with a natural log transformation. Across the full sample of each dependent measure, 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. In previous ReSource publications, data has been analyzed as change scores (e.g. 41). However, change scores can only be computed if a set of consecutive measures is available. Because the number of missing samples was larger than usual for HC and HE (Table 1), we chose to analyze the data as simple scores to be able to use all available samples.

Significance testing. All statistical analyses were conducted in the statistical software R (version 3.5.1, 57) and with an α-threshold of ≤ 0.05. Hypotheses were tested by means of multivariate linear mixed models (LMMs), which are robust to unbalanced and incomplete data in longitudinal designs. Models were fit using the function “lmer” of the r package “lme4” (58). In models predicting HC or HE, age and sex were included as covariates to account for their potential influence on hormone concentrations (24). The full model included the following terms:

$$\mathrm{DV}_{ij} = ß_{0} + ß_{1}{*}\mathrm{age}_{i} + ß_{2}{*}\mathrm{sex}_{i} + ß_{3\text{-}5}{*}\mathrm{cohort}_{i} + ß_{6\text{-}8}{*}\mathrm{timepoint}_{j} + ß_{9\text{-}13}{*}\mathrm{cohort}_{i}{*}\mathrm{timepoint}_{j} + \mathrm{rand(ID)},$$

where DV = dependent variable (cortisol, cortisone or subjective stress scores assessed via PSS and TICS), *ß0* = intercept, i = subject ID, j = measurement timepoint (T0, T1, T2, T3), rand(ID) = random intercept per subject.

In an omnibus test, we first evaluated whether the respective dependent variable differed as a function of training routine or of time, by testing for an interaction of training by time. Full models with the above outlined terms were compared with reduced models lacking the interaction term via likelihood ratio tests (59). If TCs differed from the RCC over time, the interaction model provided a significantly better fit. To ensure accurate model comparisons, models were fitted with the maximum likelihood (ML) method. Effect sizes of significant interactions were calculated as omega squared (ω^2) by dividing the variance of the residuals of the full model by the variance of the residuals of the reduced model, and subtracting the outcome from 1 (60). The resulting effect sizes were classified as small ($\omega^2 \geq .010$), medium ($\omega^2 \geq .059$) or large ($\omega^2 \geq .138$) (61). Given a significantly better fit of an interaction model, potential differences between training modules and individual measurement timepoints were evaluated in detail by contrasting model estimates through follow-up t-tests, computed through the function ‘lsmeans’ of the package ‘lsmeans’. To this end, models were re-fitted with the restricted maximum likelihood (REML) method to obtain unbiased model estimates. Follow-up contrasts were thus conducted within the LMM framework and not corrected for multiple comparisons. To assess the general efficacy of a training module, measures of stress-load following that module were compared within-subjects to the pre-training baseline, as well as between-subjects to the same testing interval in the RCC (3, 6 or 9 months). Within-subject contrasts provide a particularly sensitive assessment of change while controlling for implicit covariates, whereas between-subject comparisons are crucial to evaluate training-related change in measures with potential retest effects, which in the present study are the self-report measures. Assessment of differential training effects was conducted following the same procedure but within and across training cohorts. The results of model residual checks are reported in the Supplementary Material (Supplementary Results B, http://links.lww.com/PSYMED/A759).

Power analysis. Since the present study is part of a large-scale investigation (the ReSource Project) with numerous sub-projects, the sample sizes of the cohorts could not be tailored to this study. To determine whether the analyses planned here were sufficiently powered to be meaningful, we used the function ‘powerSim’ from the package simr (62) to simulate what effect sizes they were sensitive to, given our sample size. Power analyses were based on 1000 runs and conducted considering our hypotheses, meaning effects were simulated after Presence, Affect and/or Perspective modules. Depending on the exact pattern of effects, sufficient power was given to detect a minimum of 19-34% change in HC, 11-22% in HE, 11-21% in PSS and 13-

21% in TICS as a function of training (Supplementary Material, Table S4, http://links.lww.com/PSYMED/A759). While there are no previous studies that may serve as guidelines for reasonable effect sizes regarding HE or HC reduction after mental training, we had previously detected large relative decreases in acute cortisol reactivity of 32-59% following the same training as employed here (48). Our analyses were adequately powered to detect effects even at the lower end of that spectrum.

Baseline-matched analysis. In randomized clinical trials, baseline differences are by definition the product of chance rather than representing a latent confound (63). It is, however, possible that participants with higher baseline values are disproportionally assigned to the training cohorts by chance, leading to an overestimation of training effects through conflation with regression to the mean. Following up on our planned analyses, we examined to what extent such a pattern may have influenced study outcomes. To this end, we selected a subsample of participants with matched baseline characteristics and tested whether our results would hold in these data. Similar to clinical studies in which patients are matched to control participants based on their baseline characteristics, we here matched TC participants to RCC participants with respect to their baseline glucocorticoid levels and sex, using the function 'matchit' of the R package 'Match.It' with replacement (64). Each TC was matched separately, with the respective cohort serving as the subject pool from which participants could be drawn multiple times. Participant samples were not artificially duplicated in this process, but instead, the relative matching frequency of each participant was recorded as a weight (higher weights representing multiple matching). Weights for RCC participants were set to 1. Unmatched participants were excluded from the analysis; participants who had missing samples at baseline but provided data at a later timepoint were included. Analysis of HC and HE in this generated sample was repeated as for the main analysis, with the addition of a weighting parameter based on the frequency of matching.

Results

Of 332 participants recruited for the ReSource project, 227 provided samples of HC or HE, and 326 provided subjective stress ratings at one or more of the four measurement timepoints (see Table 1 for samples and demographic characteristics; Figure 2 and Table S1 for sample size and reasons for missingness). Participants providing hair samples were more likely to be younger and female than those who did not (Table 1 for comparisons by timepoint), and a chi-squared test of equivalence of distributions indicated that, compared to the full ReSource sample, there were marginally more women in the HC/HE subsample (χ^2=3.74, df = 1, *p*=.053). Baseline associations between dependent variables and covariates are described in Supplementary Results A, http://links.lww.com/PSYMED/A759.

Over the nine months of training, HC and HE levels showed high consistency in their pattern of change (Figure 2). A significant cohort by time interaction was detected for both HC (χ^2=30.87, df=7, *p*<.001, ω^2=0.104) and HE (χ^2=19.14, df=7, *p*=.008, ω^2= 0.036). Follow-up contrasts (Tables S5 & S6) showed that HC and HE levels remained stable in the no-training RCC. With mental training, HC and HE levels decreased steadily until six months into the training regimen, regardless of practice content (Figure 2). After three (TC3 and TC2) to six months (TC1), hair glucocorticoid levels in all training cohorts were significantly reduced compared to the respective pre-training baseline. HC concentrations at six months and HE concentrations at three to six months were lower than in the no-training RCC at the corresponding time points. Only HE in TC2 never dropped below the corresponding RCC level. At the final nine months measurement, HC and HE levels stabilized at this lowered level or regressed slightly towards baseline, but always remained significantly below baseline. Change in HC but not HE concentration was significantly and negatively associated with practice frequency (χ^2=4.46, p=.035, est.: -0.140+/-0.066, ω^2=0.025), suggesting that greater training dosage led to stronger HC reduction. Neither HC nor HE change was associated with self-reported liking of the modules.

Visualization in Figure 3 suggests that mean HC and HE baseline (T0) values differed somewhat across cohorts, with TC2 and TC3 displaying numerically higher values than TC1 and RCC. In randomized controlled trials, testing for significance of baseline differences is redundant because random subject assignment ensures that any observed baseline differences must arise by chance (63). Nonetheless, an illustrative baseline-matched weighted LMM analysis suggested that results would be comparable in a sample with matched baseline levels (Figure 4; omnibus test HC: χ^2=13.4, df=7, p=0.062, ω^2=0.082; omnibus test HE: χ^2=13.11, df=7, p=.069, ω^2= 0.032). Baseline-matched post-hoc contrasts revealed a similar pattern as in the main analysis (Figure 4). Reduced overall significance indicates a potential overestimation of training effects due to skewed baselines. Notably, however, omnibus effect sizes remained comparable to the main results (HC, main ω^2=0.104, matched: ω^2=0.082; HE, main: ω^2=0.036, matched: ω^2= 0.032), indicating that the pattern of lower significance may partially be attributable to the reduced sample size of the baseline-matched analysis, in which several TC participants were excluded due to their relatively higher hormone levels at baseline.

In another analysis of potential bias, baseline HC and HE levels did not differ between TC participants who dropped out from hair sampling during the study, and those who did not (HC: $t(112)=0.5$, $p=.62$; HE: $t(125)=-0.7$, $p=.49$), demonstrating that there was no selective drop-out.

As an exploratory outcome, potential effects of training on HC/DHEA ratios were evaluated using the same statistical approach as for the main analyses. Full to reduced model comparison showed a significant effect of the cohort by time interaction term ($\chi^2=23.17$, df=7, $p=.002$, $\omega^2=0.080$). Like the pattern observed in HC and HE, HC/DHEA ratios appeared stable in the RCC and showed decreases in TC2 and TC3 (Figure 5). HC/DHEA ratios of the TC1, however, did not decrease. Results of post-hoc comparisons are shown in Figure 5 and Table S7. Notably, a follow-up analysis of log-transformed and winzorised DHEA values independent of HC showed no significant change as a function of training ($\chi^2=9.10$, df=7, $p>.2$), suggesting that the observed change in HC/DHEA ratios may be predominantly driven by training effects on HC levels.

In the analysis of subjective-psychological stress reduction, the cohort by time interaction was significant for PSS ($\chi^2=22.20$, df=7, $p=.002$, $\omega^2=0.030$), but only marginal for TICS values ($\chi^2=13.66$, df=7, $p=.058$, $\omega^2=0.018$) (Figure 6). Follow-up contrasts of PSS scores suggested that participants reported lowest subjective stress experience following the Perspective module, but only in TC1. Exploratory LMM analyses of all samples showed no significant association between PSS or TICS scores with HC or HE concentrations throughout the study.

Similar to HC, PSS change was negatively associated with practice frequency (χ^2=4.99, *p*=.025, est.:-0.591+/-0.264, ω^2=0.010), and additionally with liking of the modules (χ^2=9.34, *p*=.002, est.:-0.975+/-0.318, ω^2=0.019; see also Supplementary Methods, http://links.lww.com/PSYMED/A759). However, the effect of practice duration disappeared when controlling for participants' ratings of how much they liked the respective module, suggesting that module enjoyment was the latent driver of the practice association. The effect of liking contrarily persisted even when controlling for practice frequency (χ^2=6.07, *p*=.014, est.:-0.815+/-0.330, ω^2=0.012). Considering that change in HC was not associated with self-reported liking, measures of stress and training engagement appear to cluster in subjective self-report measures, perhaps reflecting the lack of psychoendocrine covariance that is commonly reported in the stress literature (12,65)

Discussion

The present investigation examined whether up to 9-month long training of different types of contemplative mental practice affects physiological indices of chronic stress. Our results show that daily mental training over 3-6 months can buffer the long-term systemic stress load of healthy adults, reflected in a reduction of cortisol (HC) and cortisone (HE) accumulation in hair, and decreases self-reported chronic stress measures less consistently. This effect was independent of specific training content, positively associated with practice frequency for HC, and reached a ceiling after six months of training. It equally took six months until significant differences to baseline were achieved in all training cohorts, suggesting that reliable long-term benefits in HPA axis activity emerge only after a relatively long period of intense training. This may explain why previous studies found no HC reduction after the typical 8-12 weeks of mindfulness-based training (e.g. 32,34), with the exception of one pilot study with 18 smokers (41). Exploration of HC/DHEA ratio change revealed a similar, albeit less consistent pattern. Since DHEA alone did not change as a function of training, effects on HC/DHEA ratios were likely driven by change in HC. These results provide supporting evidence that training effects specifically affected glucocorticoid steroid hormones.

In an earlier ReSource Project publication with the same participant sample (48), we found that Affect and Perspective training selectively reduced acute salivary cortisol release in response to a stressful psychosocial laboratory challenge, the Trier Social Tress Test (TSST; 51). This differentiated pattern of results between indices of acute compared to chronic HPA axis activity suggests that distinct processes may underlie change in either type of activity. It is conceivable that stress "immunization" to a psychosocial challenge is best achieved with a training that targets social processes, such as the dyadic partner exercises implemented in the Affect and Perspective modules. In contrast, the cumulative HPA axis load as monitored in hair may reflect the more low-grade and continuous strain inherent to various daily hassles (67–69), which appears to be equally buffered by all three mental training techniques. While in the ReSource Project, we find differential training effects of the three realized practice types on many levels of observation (9), some changes seemingly need time to develop, irrespective of practice type (see also 55).

Changes in self-reported measures of chronic stress were unrelated to changes in HC and HE. This lack of psychoendocrine covariance is a recurring phenomenon in stress research (e.g. 12,50) and may be particularly emphasized through biases in retrospective self-assessments (71).

A substantial proportion of variance in hair glucocorticoids is also attributable to variables besides subjective stress, such as an individuals' general propensity to release glucocorticoids (72) as is the case for most physiological correlates of stress. While covariance can generally be improved with time-sensitive analysis techniques (73), physiological and self-report measures in the present study were retrospective in nature, precluding time-dependent dynamic analyses. The fact that integrative markers like HC do not capture time-sensitive dynamics may generally reduce psychoendocrine covariance, contributing to the pattern of poor correspondence (24,74), despite relatively consistent reports of elevated HC in highly stressed or burdened groups (24,72,73; 74 specifically find correspondence within burdened groups). As a promising remedy, one recent study was able to predict HC in healthy adults through a combination of more objective self-report data, namely counts of daily hassles, and advanced statistical modelling of time courses in subjective stress (72).

While we expected to see a decrease in subjective stress, perhaps even exaggerated through biases, change in self-report measures was inconsistent and did not show the robust reductions reported in previous studies (7). The discrepancy between TC1 and TC2 in particular suggests that the detailed pattern of change should not be overinterpreted. It is possible that participants experienced the uniquely large-scale testing of the ReSource Project as straining, leading to this discrepancy. To this effect, we previously found that the realized training practices can also be experienced as effortful (78).

Despite our large number of participants, the number of dropouts from the hair glucocorticoid assessment - partly attributable to the optional nature of this assessment - is a limitation of the current work. Importantly, however, the impact on within-cohort comparisons is limited because participants dropped out already at baseline and subsequent drop-outs were unrelated to participants' HC and HE levels. Nonetheless, results should be interpreted in the context of the specific subsamples, since participants providing hair samples were systematically younger and more female than those who did not, presumably because older men were more likely to have short hair or be bald. For future studies as well as the interpretation of this work, it should also be noted that cumulative indices of HPA-axis regulation like HC and HE do not allow specific conclusions about the physiological mechanisms leading to cortisol or cortisone levels in hair. Changes in diurnal cortisol dynamics, cortisol release under acute stress or under more low-level strain may all contribute to lower HC or HE levels. Crucially, the influence of cortisol release during eustress, such as exercise, on HC and HE also remains poorly understood (79). Future studies will need to develop time-sensitive models of how psychosocial stress and different forms of daily cortisol secretion relate to glucocorticiod accumulation in hair.

In sum, the present investigation provides evidence that mental training has a beneficial effect on individuals' long-term physiological stress load, irrespective of specific practice type. With HC and HE, we targeted the cumulative burden of frequent HPA axis activation, which is particularly maladaptive and related to ill-health. Our results thus point to one mechanism via which mental training can exert positive effects on practitioners' health status in general: By lowering systemic cortisol exposure, regular practice of about 30 minutes daily for three to six months may reduce vulnerability for stress-associated disease. We conclude that to achieve chronic stress reduction at the level of HPA axis activation, it is worth to practice more, and to carry on mental practice beyond the typical eight-week training period of mindfulness-based stress reduction programs currently offered in Western societies.

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Data availability. The present work is based on personal and sensitive physiological data that could be matched to individuals. Participants did not consent to data-sharing with parties outside the MPI CBS, such that in line with the GDPR, data cannot be made publicly available. Data are available upon reasonable request (contact via puhlmann@cbs.mpg.de).

Additional Information. Supplementary information accompanies this paper.

Author contributions. T. Singer. initiated and developed the ReSource Project and secured all funding except for the hair glucocorticoid analysis. T. Singer and V.E. designed the experiment. V.E., L.P., P.V. and R.L. were involved in data curation; L.P. and P.V. analyzed the data. C.K. funded the hair analyses. C.K. and T. Stalder were responsible for planning, performing and interpreting the hair glucocorticoid analysis. V.E. and L.P. drafted, and all authors critically revised the manuscript.

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List of Figure captions.

***Figure 1.* Study protocol and design.** A) Core processes and practices of the ReSource training. The Presence module aims to train attention and interoceptive body awareness; its two core practices are Breathing Meditation and Body Scan. The Affect module targets social emotions such as compassion, loving kindness, and gratitude; core practices are Loving-kindness Meditation and Affect Dyad. In the Perspective module, metacognition and perspective-taking on self and others are trained through the core practices Observing-thoughts Meditation and Perspective Dyad. B) Design and timeline of the Resource Project. Two training cohorts, TC1 and TC2, started their training with the mindful attention-based Presence module. They then underwent the social Affect and Perspective modules in different orders. The total training time for TC1 and TC2 was 39 weeks (13 weeks per module). TC3 only trained the Affect module for 13 weeks, and the two RCC completed all the testing without training (for more detailed information see 78). Figure adapted from (8). RCC, retest control cohort; TC1-3, training cohorts 1-3.

***Figure 2.* Participant flow chart** for analysis of HC and HE. This figure combines numbers from two recruitment periods in 2012/2013 and 2013/2014. fMRI denotes functional magnetic resonance imaging; SCID, Structural Clinical Interview for DSM-IV Disorders (Axis I and Axis II); RCC, retest control cohort; and TC, training cohort. Adapted from (50). Further detail on the gender distribution in drop-outs and final analysis samples are shown in Table S2. HC, hair cortisol; HE, hair cortisone.

[a)] Reasons for no hair sampling throughout were baldness or opting-out.

Figure 3. **Training effects on HC and HE.** Estimated A) HC and B) HE levels were derived from the linear mixed model analysis as a function of training cohort and timepoint. Note the natural log scale. Error bars represent +/- 1 SE, each circle represents one raw data point with outliers winsorized as described in the methods section. Asterisks below bars indicate comparison to RCC at the matched timepoint. *: significant at p <=.05; **: significant at p <=.01; ***: significant at p <=.001. See Tables S5 and S6 for a full list of contrast outcomes. HC denotes hair cortisol; HE, hair cortisone; SE, standard error; RCC, retest control cohort; TC, training cohort.

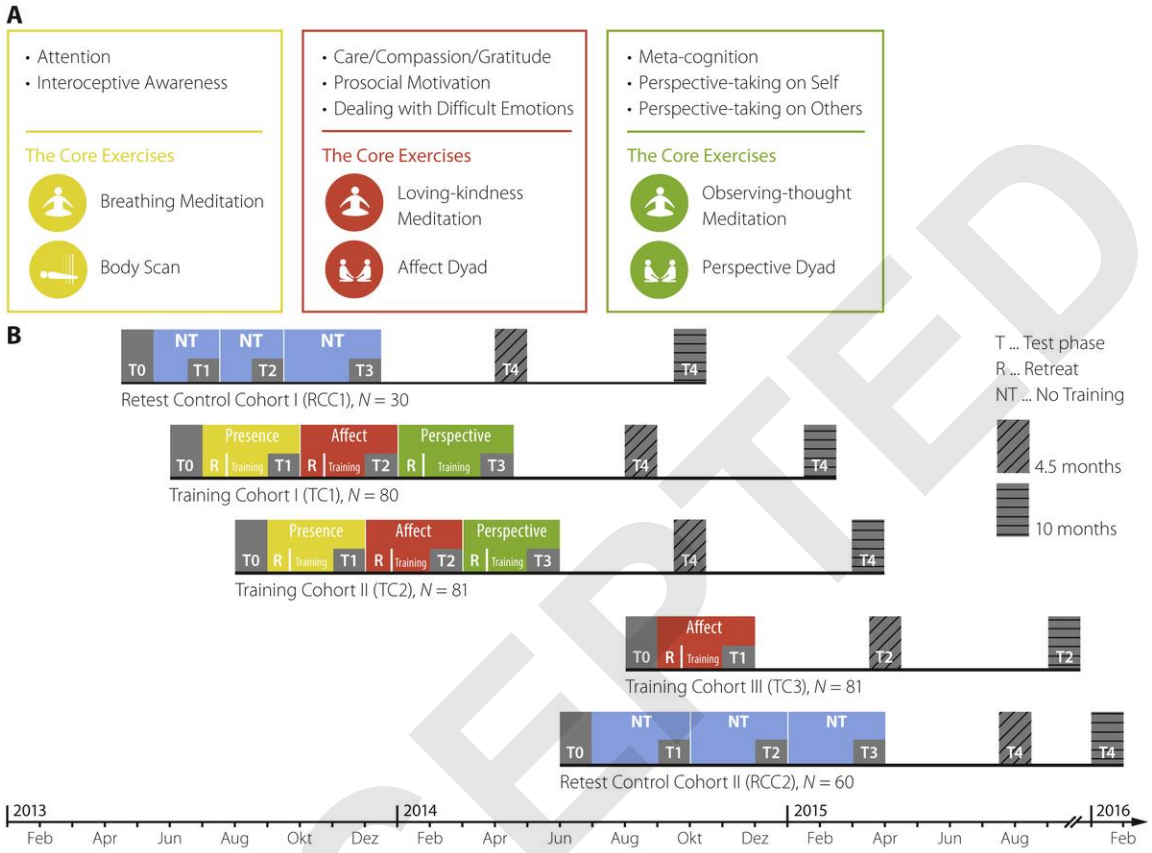
Figure 4. **Training effects on HC and HE in baseline-matched analysis.** Estimated A) HC and B) HE levels were derived from LMM analysis in a sample of participants with matched baseline HC and HE levels across cohorts, generated based on the study participant pool. Participants from each TC were matched to RCC participants with replacement depending on their baseline glucocorticoid levels and gender. Note the natural log scale. Error bars represent +/- 1 SE. Asterisks below bars indicate comparison to RCC at the matched timepoint. *: significant at p <=.05; **: significant at p <=.01; ***: significant at p <=.001. HC denotes hair cortisol; HE, hair cortisone; SE, standard error; RCC, retest control cohort; TC, training cohort.

Figure 5. **Training effects on HC/DHEA ratios in hair.** Estimated HC/DHEA ratios were derived from the linear mixed model analysis as a function of training cohort and timepoint. Note the natural log scale. Error bars represent +/- 1 SE, each circle represents one raw data point with outliers winsorized as described in the methods section. Asterisks below bars indicate comparison to RCC at the matched timepoint. *: significant at p <=.05; **: significant at p

<=.01; ***: significant at *p* <=.001. See Table S7 for a full list of contrast outcomes. HC denotes hair cortisol; DHEA, dehydroepiandrosterone; SE, standard error; RCC, retest control cohort; TC, training cohort.

Figure 6. **Training effects on self-reported long-term stress.** Estimated scores of A) Perceived Stress Scale (PSS; 26) and B) Trier Inventory for Chronic Stress (TICS; 27) derived from the linear mixed model analysis as a function of training cohort and timepoint. Error bars represent +/- 1 SE, each circle represents one data point. *: significant at p <=.05; **: significant at p <=.01; ***: significant at p <=.001.

Figure 1


A
• Attention
• Interoceptive Awareness
The Core Exercises
Breathing Meditation
Body Scan
• Care/Compassion/Gratitude
• Prosocial Motivation
• Dealing with Difficult Emotions
The Core Exercises
Loving-kindness Meditation
Affect Dyad
• Meta-cognition
• Perspective-taking on Self
• Perspective-taking on Others
The Core Exercises
Observing-thought Meditation
Perspective Dyad
B
NT
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T3
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T4
Retest Control Cohort I (RCC1), N = 30
Presence
Affect
Perspective
T0
R
Training
T1
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Training
T2
R
Training
T3
T4
T4
Training Cohort I (TC1), N = 80
Presence
Affect
Perspective
T0
R
Training
T1
R
Training
T2
R
Training
T3
T4
T4
Training Cohort II (TC2), N = 81
Affect
T0
R
Training
T1
T2
T2
Training Cohort III (TC3), N = 81
NT
NT
NT
T0
T1
T2
T3
T4
T4
Retest Control Cohort II (RCC2), N = 60
T ... Test phase
R ... Retreat
NT ... No Training
4.5 months
10 months
2013
Feb
Apr
Jun
Aug
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Dez
2014
Feb
Apr
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2015
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Feb

Figure 2

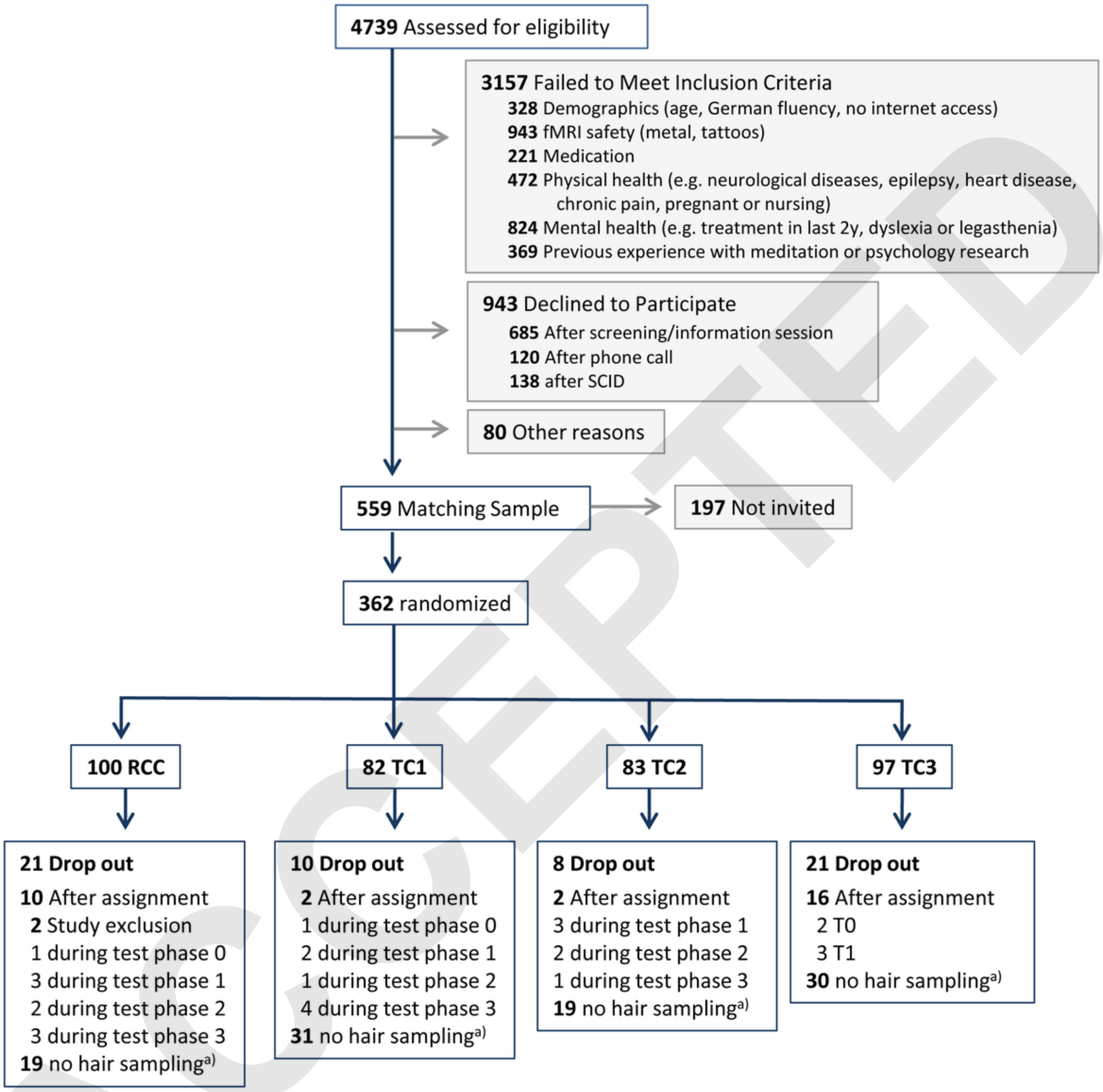
4739 Assessed for eligibility
3157 Failed to Meet Inclusion Criteria
328 Demographics (age, German fluency, no internet access)
943 fMRI safety (metal, tattoos)
221 Medication
472 Physical health (e.g. neurological diseases, epilepsy, heart disease, chronic pain, pregnant or nursing)
824 Mental health (e.g. treatment in last 2y, dyslexia or legasthenia)
369 Previous experience with meditation or psychology research
943 Declined to Participate
685 After screening/information session
120 After phone call
138 after SCID
80 Other reasons
559 Matching Sample
197 Not invited
362 randomized
100 RCC
82 TC1
83 TC2
97 TC3
21 Drop out
10 After assignment
2 Study exclusion
1 during test phase 0
3 during test phase 1
2 during test phase 2
3 during test phase 3
19 no hair sampling a)
10 Drop out
2 After assignment
1 during test phase 0
2 during test phase 1
1 during test phase 2
4 during test phase 3
31 no hair sampling a)
8 Drop out
2 After assignment
3 during test phase 1
2 during test phase 2
1 during test phase 3
19 no hair sampling a)
21 Drop out
16 After assignment
2 T0
3 T1
30 no hair sampling a)

Figure 3

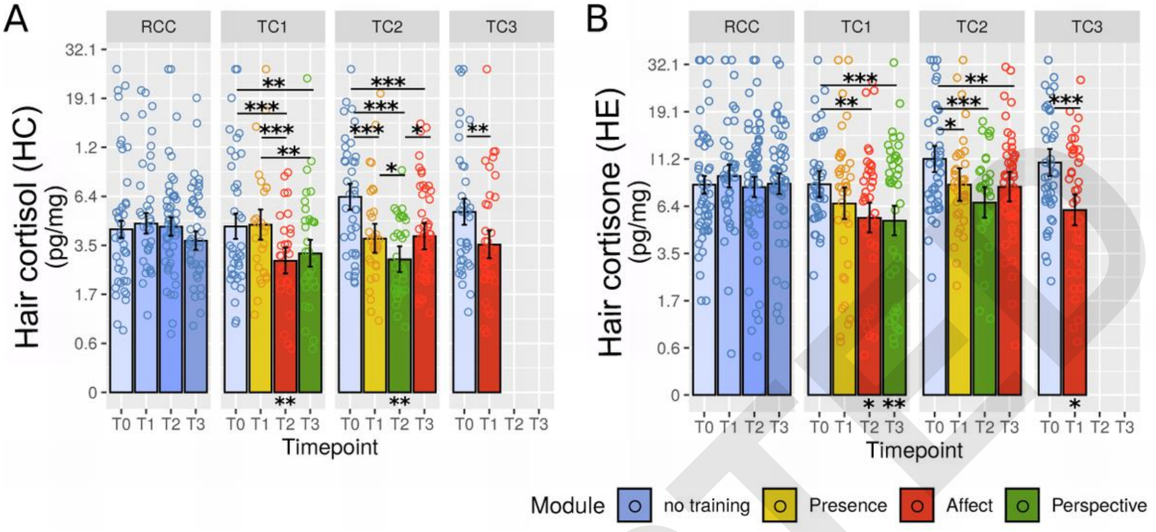
A
RCC
TC1
TC2
TC3
Hair cortisol (HC)
(pg/mg)
32.1
19.1
1.2
6.4
3.5
1.7
0.6
0
**
***
***
**
***
***
***
*
*
**
**
**
T0 T1 T2 T3
Timepoint
B
Hair cortisone (HE)
(pg/mg)
32.1
19.1
11.2
6.4
3.5
1.7
0.6
0
***
**
**
***
*
***
* **
*
Module
no training
Presence
Affect
Perspective

Figure 4

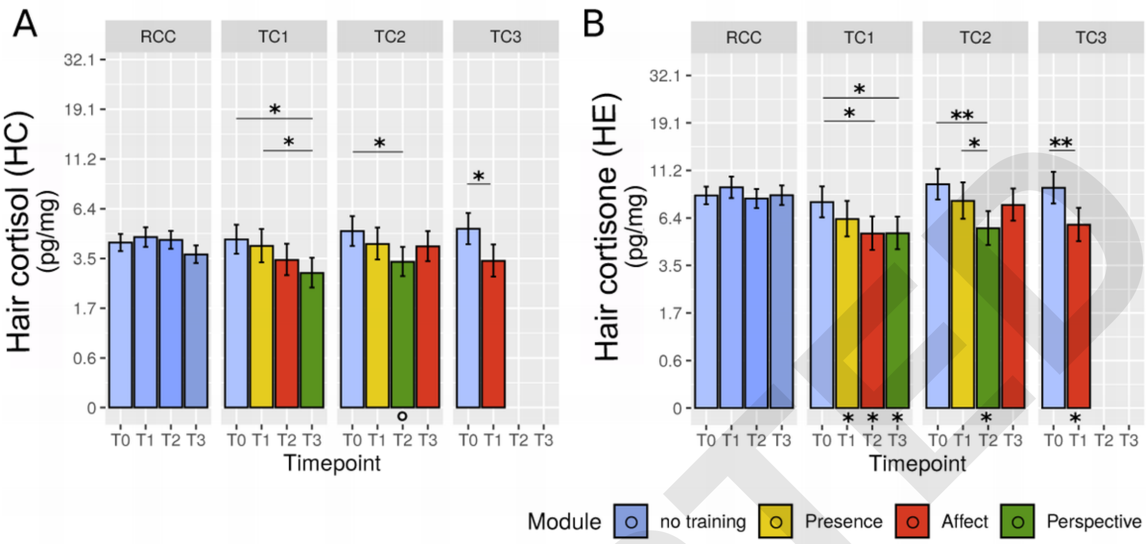
A
B
RCC
TC1
TC2
TC3
Hair cortisol (HC)
(pg/mg)
Hair cortisone (HE)
(pg/mg)
32.1
19.1
11.2
6.4
3.5
1.7
0.6
0
T0 T1 T2 T3
Timepoint
*
**
°
Module
○ no training
○ Presence
○ Affect
○ Perspective

Figure 5

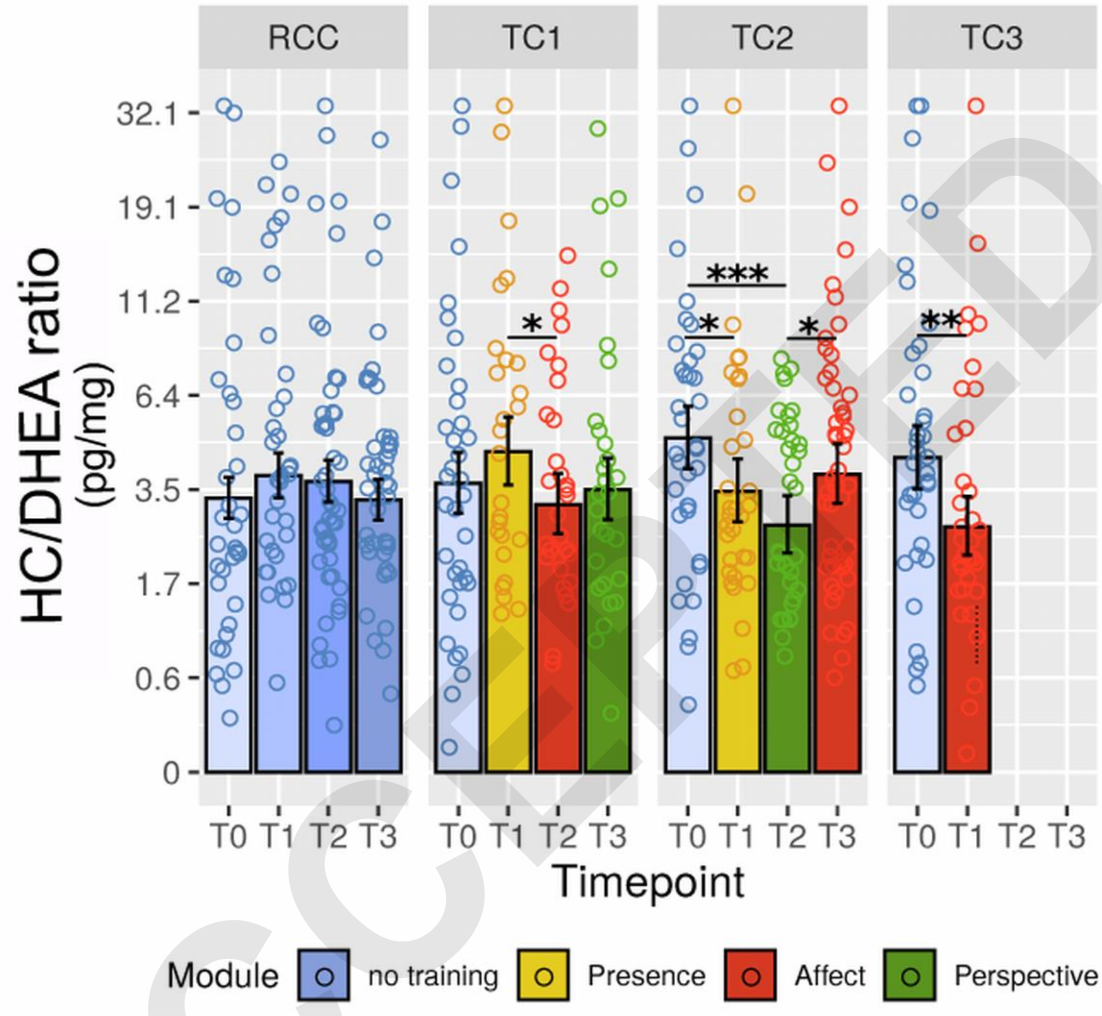
RCC
TC1
TC2
TC3
HC/DHEA ratio
(pg/mg)
32.1
19.1
11.2
6.4
3.5
1.7
0.6
0
*
***
*
*
**
T0 T1 T2 T3
T0 T1 T2 T3
T0 T1 T2 T3
T0 T1 T2 T3
Timepoint
Module
no training
Presence
Affect
Perspective

Figure 6

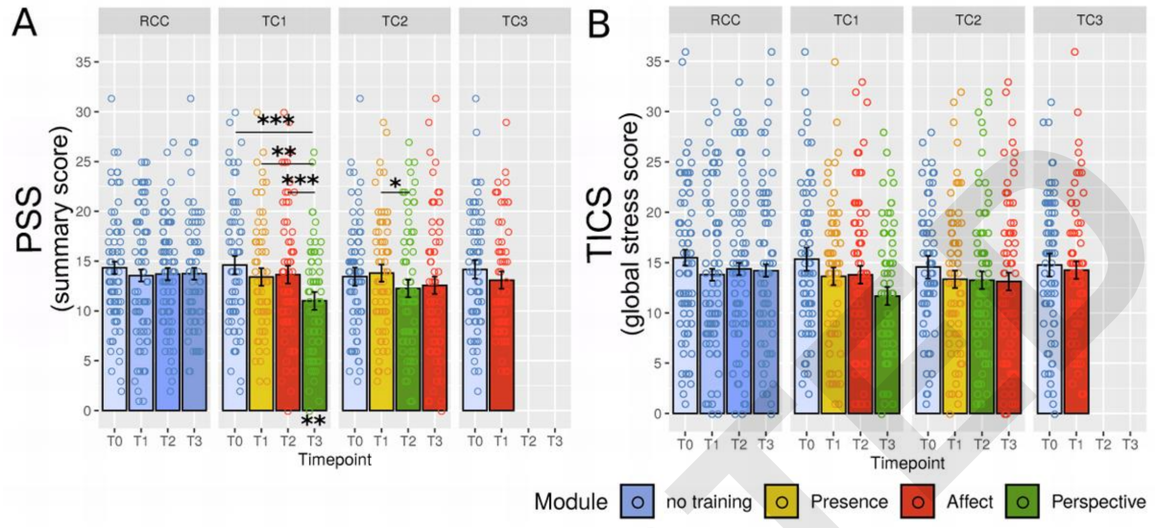
A
RCC
TC1
TC2
TC3
PSS
(summary score)
35
30
25
20
15
10
5
0
***
**
***
**
*
T0
T1
T2
T3
Timepoint
B
TICS
(global stress score)
Module
no training
Presence
Affect
Perspective

Table 1. **Raw data and demographic characteristics of samples.**

		T0	**T1**	**T2**	**T3**
HC (pg/mg)	mean (SD)	7.46 (8.97)	5.81 (6.85)	4.59 (5.18)	4.66 (3.51)
HE (pg/mg)	mean (SD)	11.6 (8.84)	9.89 (8.52)	9.03 (6.32)	9.59 (6.84)
HC/DHEA (ratio)	mean (SD)	7.85 (13.21)	6.07 (7.83)	4.77 (5.28)	5.49 (6.27)
PSS (summary score)	mean (SD)	14.1 (5.9)	13.4 (5.9)	13.2 (6.0)	12 .5 (6.1)
TICS (global stress score)	mean (SD)	15.0 (6.9)	13.8 (7.4)	13.8 (7.8)	13.1 (7.7)
HC/HE sample	N (% female) [comparison to sample without HC/HE measures]	177 (68.4% f) [χ^2=26.5, df=1, p<.001]	155 (63.2% f) [χ^2=3.35, df=1; p=.07]	131 (59.5% f) [χ^2=0.08, df=1, p>.5]	146 (64.4% f) [χ^2=9.8, df=1, p=.002]
	mean age (SD) [comparison to sample without HC/HE measures]	39.6 (9.35) [*t*(330)= 2.42, p=.016]	39.1 (9.49) [*t*(330)= 3.13, p=.002]	39.4 (9.71) [*t*(249)= 2.64, p=.009]	39.7 (9.74) [*t*(249)= 2.39, p=.018]
	smoker n (%)	21 (11.9%)	12 (7.7%)	11 (8.4%)	15 (10.3%)
PSS/TICS sample	N (% female)	322 (59.6% f)	311 (59.5% f)	233 (59.2% f)	226 (58.4% f)
	mean age (SD)	40.7 (9.22)	40.7 (9.27)	40.6 (9.35)	40.6 (9.45)
	smoker n (%)	38 (11.8%)	37 (11.9%)	30 (12.9%)	28 (12.4%)

HC/DHEA subsample	N (% female)	143 (65.0% f)	126 (59.5% f)	108 (53.7% f)	121 (62.0% f)
	mean age (SD)	39.4 (9.30)	38.4 (9.53)	38.3 (9.91)	39.1 (9.81)
	smoker n (%)	18 (12.6%)	9 (7.1%)	9 (8.3%)	12 (9.9%)

"HC/HE sample" refers to participants with at least one usable sample of either HC or HE at the given timepoint; "TICS/PSS sample" refers to participants with one or more self-report rating; "HC/DHEA subsample" refers to the subsample of participants with both HC and DHEA data. More older men than women had short hair or were bald, presumably leading to the higher % of women in the HC/HE sample. Statistical analysis confirmed that participants providing hair samples were younger and more female than those who did not; however, they did not differ on PSS or TICS scores at any timepoint. HC denotes hair cortisol, HE, hair cortisone; HC/DHEA, hair cortisol to dehydroepiandrosterone ratio; PSS, Perceived Stress Scale; TICS, Trier Inventory for Chronic Stress, f, female; SD, standard deviation. For further details on the demographic characteristics of the sample see Singer et al., 2016. Baseline associations are described in Supplementary Results A.