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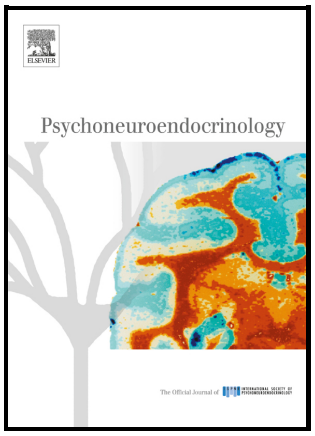

PII: S0306-4530(22)00075-0

DOI: https://doi.org/10.1016/j.psyneuen.2022.105734

Reference: PNEC105734

To appear in: *Psychoneuroendocrinology*

Received date: 2 July 2021
Revised date: 15 March 2022
Accepted date: 16 March 2022

Please cite this article as: Katja Hoehne, Pascal Vrtička, Veronika Engert and Tania Singer, Plasma oxytocin is modulated by mental training, but does not mediate its stress-buffering effect, *Psychoneuroendocrinology,* (2021) doi:https://doi.org/10.1016/j.psyneuen.2022.105734

Plasma oxytocin is modulated by mental training, but does not mediate its stress-buffering effect

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Abstract

Based on its role in social processing and stress, oxytocin has been suggested to mediate stress reduction of socio-affective, compassion-based mental training. We tested this hypothesis in the ReSource Project, a 9-month longitudinal mental training study. Participants practiced three different types of mental training, targeting either attentional abilities (Presence Module), socio-affective or socio-cognitive abilities (Affect and Perspective Modules). We investigated plasma oxytocin levels, and their link to cortisol and subjective reactivity to acute psychosocial stress as a function of previous mental training (n = 313). In a subsample (n = 113), to better understand oxytocin's involvement in the effects of socio-affective training, we explored oxytocin, cortisol and subjective experiential responses to a single Loving-kindness Meditation (LKM) conducted after three months of Affect training (versus rest without prior training). We found that, independent of mental training, stress triggered *acute* oxytocin release. Following a single LKM, however, acute oxytocin release was unaffected. Training effects were only found in *overall* oxytocin release during both, stress and LKM. Compared to no training, 3-month compassion-based Affect training decreased overall oxytocin levels in the context of psychosocial stress, but increased overall oxytocin levels during LKM. Training-induced changes in overall oxytocin were unrelated to cortisol and subjective stress reactivity. Based on Quintana and Guastella's (2020) theory of oxytocin as an allostatic hormone with anticipatory properties, we interpret training-induced changes in overall oxytocin levels as alterations in the anticipated emotional relevance of specific events. After training socio-affective skills for three months, the stressful situation may have lost its emotional saliency, whereas the meditation technique itself gained emotional relevance. We conclude that changes in peripheral oxytocin release do not mediate

stress reduction after mental training, and encourage the investigation of an allostatic concept of oxytocin in future research.

Keywords:

oxytocin, cortisol, psychosocial stress, contemplative mental training, meditation

1 Introduction

Contemplative mental practice is a popular method to reduce stress, and promote health and wellbeing. Mindfulness-based interventions, such as the Mindfulness-Based Stress Reduction Program (Kabat-Zinn, 1994), have even built a strong reputation in mainstream clinical and educational settings (Davidson and Kaszniak, 2015). Besides mindfulness-based interventions, which cultivate moment-to-moment awareness of internal and external events, compassion-based interventions, such as Compassion Focused Therapy (Gilbert, 2009), foster positive affect and social abilities. We and others have hypothesized, that the beneficial effects of particularly compassion-based mental training may be mediated through increased availability of the neuropeptide oxytocin (Engert et al., 2017; Mascaro et al., 2015). Focusing on training-induced changes in stress sensitivity, we investigated this hypothesis within the ReSource Project, a 9-month longitudinal mental training study (Singer et al., 2016).

Oxytocin is known for its role in affiliation, bonding (Insel and Young, 2001), social cognition, and social affect (McCall and Singer, 2012; Winslow and Insel, 2004), and therefore a prime candidate to respond to compassion-based interventions. Accordingly, after intranasal oxytocin application, healthy participants exhibited enhanced empathy (e.g. Bartz et al., 2019; Hurlemann et al., 2010; but see Singer et al., 2008), and were better able to imagine compassionate qualities (e.g. Rockliff et al., 2011; although, depending on individual differences, this effect was experienced as more or less agreeable). Moreover, higher blood oxytocin levels (from unextracted plasma) were associated with higher empathy ratings in

healthy participants after watching emotional videos (e.g. Barraza and Zak, 2009). However, oxytocin is more than a "social hormone", and is involved in learning and memory, food intake, sexual behaviour, aggression, pain, anxiety, and stress, among others (Jurek and Neumann, 2018).

Stress refers to a state in which adverse stimuli threaten an organism's homoeostasis. Consequently, the organism generates a compensatory response of sympatho-adrenal-medullary system and hypothalamo-pituitary-adrenal (HPA) axis activation, resulting in catecholamine and cortisol release. This active process is termed allostasis, which means attaining stability through change (Chrousos, 2009; McEwen, 2008). A job interview, social rejection or financial strain are examples for the psychosocial stressors we face daily, often for months on end, in modern societies. While an acute stress response is adaptive, chronic stress exposure may result in the accumulation of allostatic load, leading to an increased risk for stress-related diseases (Chrousos, 2009; Sapolsky, 2015).

A wealth of literature suggests a stress-dampening function of oxytocin. Human studies in breastfeeding women with elevated endogenous oxytocin levels (Heinrichs et al., 2001; Light et al., 2000), and using intranasal oxytocin application (e.g. Heinrichs et al., 2003; Kubzansky et al., 2012; Quirin et al., 2011) revealed attenuated cortisol release and blood pressure, changes in heart rate variability, and anxiolytic effects following psychosocial stress. In one study, the stress-dampening role of oxytocin was reflected in a negative association of peripheral oxytocin and cortisol levels, both averaged across the duration of a psychosocial stress paradigm (Pierrehumbert et al., 2010). Interestingly, when considering the temporal dynamics of the stress response, different markers exhibited opposite association patterns with peripheral oxytocin. Thus, studies showed increased peripheral oxytocin *reactivity* to psychosocial stress (e.g. Bernhard et al., 2018; de Jong et al., 2015; Engert et al., 2016; Light et al., 2000; Pierrehumbert et al., 2010), which correlated positively with stress-

induced cortisol release (Bernhard et al., 2018; de Jong et al., 2015; Engert et al., 2016). Conversely, during subsequent *recovery*, stress-induced oxytocin release was linked to faster recovery of cortisol (Bernhard et al., 2018) and heart rate variability (Engert et al., 2016). Attempting to consolidate its various functions, Quintana and Guastella (2019) hypothesized oxytocin to be an allostatic hormone that regulates social and non-social behaviour to preserve the organism's stability through changing environments, and to promote survival. The above stress findings nicely support this notion by showing that, first, oxytocin quickly reacts to fast-changing environments (i.e., stressors), and, second, enables faster recovery after stressor termination, thus preserving stability of the organism.

Contemplative mental training has an influence on both, oxytocin regulation and stress sensitivity. The effect of mental training on oxytocin levels was just recently revealed. Bellosta-Batalla et al. (2020b) reported that, compared to control groups, a two-month mindfulness and compassion-based program increased basal salivary oxytocin levels, and improved perspective-taking. Further, they found increased basal salivary oxytocin levels, and decreased anxiety and negative affect after a single mindfulness session (Bellosta-Batalla et al., 2020a). Regarding stress sensitivity, mindfulness- and compassion-based programs can reduce reactivity to acute psychosocial stress in healthy participants by lowering subjective stress (e.g. Arch et al., 2014; Engert et al., 2017; Pace et al., 2009), cortisol (e.g. Engert et al., 2017), sympathetic (e.g. Arch et al., 2014; Nyklíček et al., 2013) and immune responses (e.g. Pace et al., 2009; Rosenkranz et al., 2013). Research from our group revealed that mental practice type is an important factor to consider (Singer and Engert, 2019). Namely, cortisol release following psychosocial stress was reduced after socio-affective (compassion-based) and socio-cognitive (thought-based) training, but not after training mindfulness-based attention and interoception (Engert et al., 2017).

We here examined the effects of different contemplative mental training types on overall and psychosocial stress-induced plasma oxytocin levels. Subsequently, the role of oxytocin in stress reduction after mental training was investigated. In a second subproject, we explored oxytocin, cortisol and subjective responses to one session of Loving-kindness Meditation (LKM) after three months of compassion-based mental training compared to a rest session without prior training. Our study was conducted as part of the ReSource Project. The longitudinal ReSource training comprised three distinct 3-month modules cultivating present-moment focused attention and interoceptive awareness (Presence Module), gratitude, compassion, prosocial motivation, and dealing with difficult emotions (Affect Module), and metacognition and perspective-taking on self and others (Perspective Module; Figure 1A). In the study's conceptual phase, the available literature suggested that oxytocin enhances socio-affective processes, and dampens the stress response. Based on these initial assumptions, we expected elevated overall and stress-induced plasma oxytocin levels after the Affect Module in particular. Given training-induced changes in oxytocin levels, we hypothesized a negative association with training-induced reductions in stress reactivity. Here, we focused on subjective and cortisol stress reactivity because, in a previous study using the same participants, mental training affected specifically these parameters (Engert et al., 2017). During LKM, we expected an additional increase in oxytocin release due to the acute meditation experience. Given such an effect, a negative relation between oxytocin and the assessed stress markers was again expected. To characterize the meditation experience, we additionally explored subjective responses (arousal, valence, warmth, and effort) to LKM.

2 Materials and Methods

2.1 Participants

For the ReSource Project, 332 healthy participants (197 women, age mean ± *SD*: 40.74 ± 9.24; range: 20 to 55 years) were recruited between April 2013 and February 2015, and

assigned to one of three training cohorts (TC1: *n* = 80; TC2: *n* = 81; TC3: *n* = 81), or to a retest control cohort (RCC: *n* = 90). RCC participants were recruited either between April and December 2013 (RCC1; *n* = 30), or April 2014 and February 2015 (RCC2; *n* = 60). Participants completed a face-to-face mental health diagnostic interview with a trained clinical psychologist. The interview covered a computer-assisted German version of the structured Clinical Interview for DSM-IV Axis-I disorders, the SCID-I DIA-X (Wittchen and Pfister, 1997), and a personal interview for Axis-II disorders, the SCID-II (Wittchen et al., 1997). Exclusion criteria were any Axis-I disorder within the past two years, or schizophrenia, psychotic disorder, bipolar disorder, substance dependency, or an Axis-II disorder at any time in life. Volunteers taking medication influencing the HPA axis were excluded. For detailed information on recruitment and exclusion criteria see Chapter 7 of Singer et al. (2016). The ReSource Project was registered with the Protocol Registration System of ClinicalTrial.gov under the title "Plasticity of the Compassionate Brain" (Identifier NCT01833104), and approved by the ethic boards of Leipzig University (ethic number: 376/12-ff) and Humboldt-University Berlin (ethic numbers: 2013-20, 2013-29, 2014-10). Participants gave written informed consent, could withdraw from the study at any time, and were financially compensated.

For stress testing, 313 participants (185 women, age mean ± *SD*: 40.68 ± 9.30; range: 20 to 55 years) of the ReSource sample (*n* = 332) underwent the Trier Social Stress Test (TSST) (Kirschbaum et al., 1993). To examine oxytocin during a single LKM session versus at rest, 113 participants (62 women, age mean ± *SD*: 40.88 ± 8.76; range: 23 to 55 years) from TC3 (*n* = 67, 37 women) and RCC2 (*n* = 46, 25 women; Figure 1B) were tested (see Supplemental Material for exclusion, drop out, and descriptive statistics of both samples).

2.2 Experimental design and procedure

2.2.1 Stress session

The ReSource Project was organized in a longitudinal design (Figure 1B). The two major training cohorts TC1 and TC2 received nine months of training, experiencing the three 3-month modules in different orders. The RCC was also followed over nine months, but received no mental training. TC3 received 3-month Affect training only. Yet, stress testing was realized cross-sectionally, such that each participant completed the TSST just once, and different participant groups were tested at different stages of the ReSource Project. In total, 313 participants underwent the TSST, of whom 130 (74 women) were tested without training experience. Of these, 84 participants belonged to the RCC, and were tested at baseline (T0; n = 46), in the first testing phase (T1; n = 20), or in the second testing phase (T2; n = 18); 46 belonged to one of the training cohorts, and were tested at T0 prior to training. Of the remaining 183 training participants (111 women), 46 underwent the TSST at T1 following the Presence Module, 46 at T1 following the Affect Module, 44 at T2 following Presence and Affect Modules, and 47 at T2 following Presence and Perspective Modules.

Due to the circadian rhythm of cortisol secretion (Kirschbaum and Hellhammer, 1989), testing was conducted between 12 pm and 6 pm in one 130-min session. Upon arrival at the laboratory, participants received a standardized snack to equalize blood sugar levels in order to avoid an unsystematic influence of prior food intake. During testing, they took nothing by mouth except water. At 15 min after arrival, baseline subjective stress questionnaires and cortisol samples were collected (-55 min before stressor onset). Then, blood samples for oxytocin assessment were drawn (-50 min). To overcome potential stress effects triggered by the blood draw, a 30-min rest phase was introduced, after which participants were given the TSST testing instructions. After 15 min of stress anticipation, the subjective stress questionnaire was administered for a second time (-5 min), followed by the stress phase. Subjective stress questionnaires, saliva, and blood samples were collected after

the stressor (10 to 15 min) and throughout the 50-min recovery phase. The final blood sample was taken at 60 min after stressor onset (Figure 2A).

2.2.2 LKM and rest session

In the LKM/rest session, 113 participants were tested. For TC3 (n = 67), testing took place at T1 after three months of Affect training; for RCC2 (n = 46) at T2 without prior training (Figure 1B). Plasma oxytocin was assessed before and after a single 30-min LKM session (TC3), or a 30-min rest period (RCC2). Each testing session lasted 90 min and was performed either between 9.30 am and 1 pm, or 6.30 pm and 9 pm, depending on participant availability. Again, participants received a standardized snack upon arrival to equalize blood sugar levels. They took nothing by mouth except water during testing. Baseline samples were collected at -35 min (subjective experience), -20 min (cortisol), or -10 min (oxytocin) relative to LKM/rest onset. Prior to LKM/rest, participants completed subjective questionnaires (-5 min). They then engaged in LKM or sat comfortably in a chair while reading magazines (rest). At 35 min, subjective and cortisol samples were collected, followed by the second oxytocin blood draw (40 min). A final cortisol sample was collected at 60 min (Figure 2B).

2.3 ReSource training program

The ReSource training consists of three 3-month modules (Presence, Affect, and Perspective), each cultivating distinct socio-cognitive and socio-affective skills (Singer et al., 2016). TC1 and TC2 started their training with the attention-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, TC3 underwent a 3-month Affect training only (Figure 1B). At the beginning of each module, participants attended 3-day retreats to familiarize themselves with the practices of each module. Subsequently, they met in groups with their teachers for two hours weekly. Our

recommendation was to practice for approximately 30 min on 5 days per week using a custom-made smartphone-app or via an internet platform.

The processes targeted in the Presence Module were attention and interoceptive awareness, trained through the core exercises Breathing Meditation and Body Scan. The Affect Module targeted the cultivation of social emotions, such as compassion, loving kindness, and gratitude, and aimed to enhance prosocial motivation and dealing with difficult emotions. The core exercises were LKM and an Affect Dyad. In the Perspective Module, participants trained meta-cognition and perspective-taking on self and others through Observing-thought Meditation and a Perspective Dyad. The dyadic format was designed to foster interconnectedness by providing opportunities for self-disclosure and mindful listening (Kok and Singer, 2017). In each 10-min dyadic practice, two participants shared their experiences with alternating roles of speaker and listener. This contemplative dialog is understood as a "loud meditation" (Figure 1A, for more information on the dyadic format see Chapter 3 in Singer et al. (2016)).

2.4 Stress induction

Participants underwent the Trier Social Stress Test (TSST; Kirschbaum et al., 1993), a standardized psychosocial stress paradigm. It comprises of an anticipation phase (15 min in this study), an audio- and videotaped mock job talk (5 min), and a difficult arithmetic task (5 min). These challenges are performed in front of a gender-mixed committee of two alleged behavioural analysts, trained to be non-empathic to the participant's struggles.

2.5 Physiological measures

2.5.1 Oxytocin

Peripheral oxytocin levels were determined from venous blood collected in 4 ml ethylenediaminetetraacetic acid (EDTA) Monovette tubes (Sarstedt, Nümbrecht, Germany). Blood samples were immediately centrifuged (5 min, 4000 rpm, 4 ˚C). Plasma was pipetted into 2 ml SafeSeal Micro tubes (Sarstedt, Nümbrecht, Germany), and stored at -30˚C until assay. Then, plasma samples were extracted using LiChro-prep Si60 (Merck). Oxytocin levels (pg/ml) were determined using a highly sensitive (0.5 pg/ml range) and specific (<0.7% cross-reactivity to a variety of peptides) radioimmunoassay with intra- and inter-assay variabilities of less than 10% (RIAgnosis, Germany). The utilized assay has been standardized and validated in numerous animal and human studies (Landgraf and Neumann, 2004).

Regarding the stress session, a first assay of samples collected at T0 was conducted in 2014 to address cross-sectional research questions before termination of the longitudinal data collection (Engert et al., 2016). For the current study, these baseline samples were re-assayed jointly with all additional samples (assessed at T1 and T2) to avoid potential systematic effects of storage time, and to minimize reagent batch effects.

2.5.2 Cortisol

As biological marker of HPA axis activity, cortisol was collected via saliva samples using Salivettes (Sarstedt, Nümbrecht, Germany). Participants placed the collection swabs in their mouth, and refrained from chewing for 2 min. Salivettes were stored at -30˚C until assay. For determination of cortisol levels (nmol/l), a time-resolved fluorescence immunoassay with intra- and interassay variabilities of less than 10 and 12 % was used (Dressendörfer et al., 1992).

2.5.3 Additional biomarkers

Additional biomarkers (alpha-amylase, heart rate, heart rate variability, interleukin-6, and C-reactive protein) were assessed before and after the TSST. Since they were unaffected

by the mental training intervention (Engert et al., 2017), there were not considered in the current analysis.

2.6 Self-report measures

2.6.1 Subjective experience during stress

During psychosocial stress, subjective experience was assessed using the 20-item state scale of the State Trait Anxiety Inventory (STAI, Spielberger et al., 1983). The STAI targets feelings of apprehension, nervousness, tension, worry, and activation/arousal of the autonomic nervous system.

2.6.2 Subjective experience during LKM/rest

Throughout LKM/rest, participants reported their feelings of stress and warmth using a single-item rating scale ranging from 1 ("not at all") to 10 ("a lot"). They also completed the Affect Grid (Russell et al., 1989), a visual single item scale to assess the dimensions of pleasure-displeasure and arousal-sleepiness. These dimensions are aligned on the x- and y-axes of a grid, in which participants mark their current state. After LKM/rest, participants reported how effortful they perceived meditation or rest to be using a rating scale ranging from 1 ("not at all") to 10 ("a lot").

2.7 Statistical analyses

2.7.1 Data preparation

All analyses were performed with the software R, version 4.0.2 (R Core Team, 2020). Due to skewness, physiological data was ln-transformed to approach normal distribution. Outliers were winsorized to 3 *SD*s from the mean. Continuous predictors (except daytime) were mean-centered to facilitate interpretation. Significance was set to a level of $p \leq 0.05$, and

all tests were two-sided. Due to unbalanced group sample sizes, type 3 sums of squares with orthogonal contrasts were reported for all *F*-statistics. As the subprojects are part of a greater project, power analysis could not be conducted for each individual subproject. However, we calculated post-hoc power analyses. These suggest that some of the training effects might be underpowered (see Table S5 in Supplemental Material). Nevertheless, our sample size is considerably bigger than typically seen in meditation studies, and studies analysing plasma oxytocin levels.

Because oxytocin levels are influenced by ovarian hormones (de Jong et al., 2015; Engert et al., 2016), hormonal status (male, no menstrual cycle, hormonal contraceptives, natural menstrual cycle) was added as a covariate to all oxytocin models. For cortisol models, hormonal status and daytime were added as covariates to control for their influence (Allen et al., 2014; Kirschbaum and Hellhammer, 1989). Daytime was also considered in all models from the LKM/rest session because participants were scheduled for testing either in the morning or evening. Given the considerable age range of our participants, age was included in all analyses.

2.7.2 Matching

ReSource participants were assigned to their cohorts matched in demographics and various self-reported traits using bootstrapping without replacement (for matching details see Chapter 7 in Singer et al. (2016)). For the cross-sectional stress testing design, groups were rematched on variables with potential influences on stress reactivity (for details see Supplemental Material).

2.7.3 Training effects on oxytocin levels during acute psychosocial stress

A linear mixed model (LMM) was used to examine mental training effects on oxytocin levels during acute psychosocial stress. Repeated measures of oxytocin were nested

within individuals. The peak in stress response (15 min after stressor onset) represented the intercept of the model, and was set to 0. Oxytocin reactivity and recovery were estimated by continuous time variables modelling the minutes between measurement time-points: from baseline to peak (-50 min to 15 min; reactivity slope), and from peak to recovery (15 min to 60 min; recovery slope). Next to the time slopes, we added the group variable (no training, Presence, Affect, Presence/Affect and Presence/Perspective), its interactions with reactivity and recovery slopes, and covariates (hormonal status, age) to the model (see Supplemental Material for the final model building). This approach allowed us to examine training effects on oxytocin stress reactivity (interaction effect: reactivity slope*group), peak (main effect: group) and recovery (interaction effect: recovery slope*group) in one single model. Significant main effects were followed up with pairwise post-hoc comparisons, corrected for multiple testing by Tukey-Kramer using a multivariate *t*-distribution. A model without non-significant interactions was used for post-hoc comparisons and the calculation of Cohen's *d*. Cohen's *d* between groups was calculated using *t* values and the number of peak samples per group.

2.7.4 Associations of changes in oxytocin levels and stress reactivity after mental training

To test for associations of training-induced changes in oxytocin levels with training-induced reductions in cortisol and subjective stress reactivity (Engert et al., 2017), change scores for each stress marker were created by subtracting the baseline from the peak measurement (cortisol peak: 20 min, STAI peak: -5 min to stressor onset). Given results from the preceding analysis (see 3.2 in the Results section), only overall oxytocin levels were considered. As a proxy of overall oxytocin levels, an area under the curve with respect to ground (AUC_g, Pruessner et al., 2003) was calculated from the three oxytocin measurements. All scores were computed with the ln-transformed and winsorized data. Cortisol change was adjusted for baseline levels by extracting the standardized change score residuals from a

regression model. Analyses of covariance (ANCOVAs) were conducted with cortisol and subjective stress reactivity change scores as dependent variables. Oxytocin AUC_g, group, their interaction, and covariates (cortisol: age, hormonal status, daytime; STAI: age, sex) were entered as predictors into the models.

2.7.5 Effect of a single LKM session on levels of oxytocin, cortisol, and subjective experience

LMMs were used to examine the effects of one LKM versus rest on levels of oxytocin, cortisol, subjective stress, arousal, valence, warmth, and effort. Repeated measures of each variable (except effort) were nested within individuals. The baseline measurement (oxytocin: -10 min relative to LKM/rest onset; cortisol: -20 min; self-reports: -35 min) represented the intercept of each model, and was set to 0. Change over time was estimated by a continuous time variable, modelling the minutes between measurement time-points from baseline to post-LKM/rest (oxytocin: 40 min; self-reports: -5 and 35 min; cortisol: 35 and 60 min). Further predictors were cohort (TC3 vs. RCC2), its interaction with the time variable, and the covariates hormonal status (sex for self-reports), daytime, and age. Again, this modelling approach allowed us to examine effects of previous mental training/no training on baseline levels of each marker (main effect: cohort), and for cohort-dependent differences in how the markers changed over the course of the meditation session/rest (interaction effect: time*cohort) in one single model. For subjective effort, which was assessed only once, an ANCOVA was realized instead. Cohen's *d* between cohorts was calculated using *t* values and considering either the number of baseline samples per cohort for the main effect of cohort, or of post LKM/rest samples per cohort for the interaction effect of time by cohort. For stress markers and oxytocin, a model without non-significant interactions was used for the calculation of Cohen's *d*.

Since TC3 and RCC2 did not differ in oxytocin responses to meditation and rest (see Results section), no associations with cortisol or subjective experience were explored.

1 3 Results

3.1 Preliminary analysis

An overview of missing data and winsorized outliers is given in Table S2. A subsistent stress response, defined as a minimal cortisol increase of 1.5 nmol/l from baseline (Miller et al., 2013), was observed in 75 % of our sample, suggesting successful stress induction.

3.2 Training effects on oxytocin levels during acute psychosocial stress

The LMM examining training effects on oxytocin levels during psychosocial stress showed that plasma oxytocin levels increased in response to the stressor ($F_{(553)}$ = 69.38, p < .001), and decreased during recovery ($F_{(553)}$ = 60.26, p < .001). No effect of mental training type was found on oxytocin reactivity and recovery. However, training groups differed significantly in their oxytocin peaks ($F_{(444)}$ = 6.35, p < .001; Table 1A and Figure 3A). Post-hoc comparisons revealed that these differences were significant between no training and Affect only groups ($t_{(282)}$ = 3.16, p < .05, d = 0.56, 95% CI [0.21; 0.92]), between Presence and Affect only groups ($t_{(281)}$ = 4.40, p < .001, d = 0.95, 95% CI [0.50; 1.39]), and between Presence and Perspective (after Presence) groups ($t_{(278)}$ = 3.07, p < .05, d = 0.66, 95% CI [0.23; 1.10]; Table 1C). The Affect only group exhibited lower oxytocin peak levels than no training and Presence groups, and the Perspective (after Presence) group exhibited lower oxytocin peak levels than the Presence group. Due to non-normal residuals, the analysis was recalculated as a robust model, revealing the same significance pattern.

Owing to the statistical modelling, the oxytocin peak (and intercept of our model) was independent of stress reactivity and recovery slopes (see 2.7.3 in the Methods section).

Therefore, we suggested that the oxytocin peak may represent a proxy of overall oxytocin release. Another accepted measure of overall hormonal output is the area under the curve with respect to ground (AUC_g, Pruessner et al., 2003). AUC_g and the oxytocin peak in our data revealed a correlation of r = .95. Hence, the above analysis was repeated using an ANCOVA with overall oxytocin levels (represented by the AUC_g) as dependent variable, and pairwise post-hoc comparisons. Post-hoc comparisons were corrected for multiple testing by Tukey-Kramer using a multivariate *t*-distribution. Results showed the same significance pattern as the above analyses (see Table S3A and S3C in Supplemental Material). Thus, a measure of overall oxytocin activity rather than acute reactivity was influenced by mental training.

3.3 Associations of changes in oxytocin levels and stress reactivity after mental training

ANCOVAs examining the link of training-induced changes in overall oxytocin release with training-induced reductions in subjective stress and cortisol during psychosocial stress (as found in Engert et al., 2017), showed no association of oxytocin AUC_g with either cortisol or subjective stress reactivity (Table 2). In other words, reductions in cortisol and subjective stress reactivity specifically after Affect, but also after Perspective training, were independent of training-induced changes in overall oxytocin plasma levels. Due to non-normality, significance patterns were confirmed by a robust model.

3.4 Effect of a single LKM on levels of oxytocin, cortisol, and subjective experience

The LMM examining training effects on oxytocin levels during one LKM versus rest session stems from a comparison of two ReSource *cohorts*, TC3 (Affect only training) and RCC2 (no training), rather than the *groups* tested in the stress models (Figure 1B). The LMM showed no change in oxytocin levels over time, and no time by cohort interaction. However, there was a main effect of cohort on oxytocin levels at baseline ($F_{(164)}$ = 6.62, p < .05, d = -

0.72, 95% CI [-1.11; -0.32]), with higher oxytocin levels in TC3 than RCC2 (Table 1B and Figure 3B).

Based on our statistical modelling, the oxytocin baseline (and intercept of our model) was independent of the oxytocin slope (see 2.7.5 in the Methods section). Therefore, we proposed that the oxytocin baseline may again represent a proxy of overall oxytocin release. To test this assumption, the oxytocin baseline and the AUC_g were correlated, revealing a correlation of r = .91. Hence, the above analysis was repeated as an ANCOVA using overall oxytocin (represented by the AUC_g) as dependent variable. Results showed the same significance pattern as the above LMM (see Table S3B in Supplementary Material). Thus, similar to the stress results, a measure of overall oxytocin activity rather than acute reactivity was influenced by preceding mental training. Other than for stress, however, Affect training was linked to increased, rather than reduced, overall oxytocin levels.

Despite the lack of an acute meditation effect on oxytocin levels, TC3 and RCC2 differed in their responses of emotional warmth, valence, and effort to either meditation or rest. Emotional warmth (d = -0.72, 95% CI [-1.12; -0.33]) and positive valence (d = -0.40, 95% CI [-0.78; -0.02]) increased after meditation (in TC3) compared to rest (in RCC2) (all $F \geq 4.44$, all $p < .05$). Also, subjective effort was rated higher after meditation than rest ($F_{(101)}$ = 36.54, $p < .001$, d = -1.18, 95% CI [-1.60; -0.77]). Baseline differences between TC3 and RCC2 revealed lower cortisol (d = 0.53, 95% CI [0.13; 0.92]), less positive valence (d = 0.54, 95% CI [0.15; 0.92]), and higher emotional warmth (d = -0.69, 95% CI [-1.08; -0.30]) and subjective stress (d = -0.54, 95% CI [-0.93; -0.16]) after 3-month Affect (only) compared to no training (all $F \geq 7.71$, all $p < .01$) (Table 3 and Figure S1 in Supplemental Material). Due to non-normality and/or heteroscedasticity (cortisol, subjective stress, warmth, valence, and effort), significant patterns were confirmed with robust models.

3.5 Comparison of overall oxytocin levels in the stress and LKM/rest session

As depicted above, overall oxytocin levels during psychosocial stress were lower in the Affect only than in the no training group. Contrarily, during the LKM/rest session, overall oxytocin levels were higher in TC3 than in RCC2 (Figure 3). To explore whether these fluctuations in overall oxytocin release between testing sessions are indeed meaningful, we calculated a dependent samples ANCOVA for overall oxytocin levels (AUC_gs) with the factors session (stress vs. meditation), group/cohort (Affect/TC3 vs. no training/RCC2), and the covariates age, hormonal status, and daytime. Because stress testing was conducted cross-sectionally (i.e., in groups independent of cohorts), while meditation testing was performed within the cohorts (TC3 and RCC2), some TC3 attendees of the LKM session were initially assigned to the no training group and tested at T0 for stress testing (Figure 1B). These participants were excluded from the current analysis, leading to a sample of n = 41 for the Affect group/TC3 cohort, and n = 46 for the no training group/RCC2 cohort. To correct for unequal numbers of oxytocin samples per session (three during stress vs. two during LKM/rest), each AUC_g was divided by the respective number of oxytocin measurements.

A significant interaction of session and group/cohort confirmed that, indeed, Affect/TC3 participants had lower overall oxytocin levels than no training/RCC2 participants during psychosocial stress, and higher overall oxytocin levels than no training/RCC2 participants during the LKM/rest session ($F_{(77)}$ = 26.46, p < .001) (see Table S4 in Supplemental Material and Figure 3C). This interaction was driven by relatively increased overall oxytocin levels in no training/RCC2 participants during psychosocial stress compared to rest ($F_{(42)}$ = 87.18, p < .001, d = -2.06, 95% CI [-2.59; -1.52]). Overall oxytocin release in Affect/TC3 participants did not differ between sessions ($F_{(36)}$ = 0.95, p = .336). Due to non-normality and heteroscedasticity, significant patterns were confirmed with robust models.

2 4 Discussion

We investigated the role of plasma oxytocin in mediating stress reduction after contemplative, specifically socio-affective (i.e., compassion-based), mental training. In the first sub-study, we examined whether different types of mental training practices (attention-based, socio-affective, and socio-cognitive) differentially influenced plasma oxytocin levels during acute psychosocial stress, and whether such changes in oxytocin levels were associated with previously observed training-induced reductions in subjective and cortisol stress reactivity (Engert et al., 2017). In the second sub-study, using a subsample of the first, we explored the effect of a single compassion-based Loving-kindness Meditation (LKM) after 3-month socio-affective training (versus rest without prior training) on levels of oxytocin, cortisol and subjective experiences of stress, arousal, valence, warmth, and effort.

Independent of preceding mental training, oxytocin levels increased in response to a laboratory stressor, and subsequently dropped back to baseline levels within one-hour post-stress. Contemplative mental training affected only *overall* oxytocin release. Before, during and after psychosocial stress, oxytocin levels were reduced after 3-month socio-affective training (Affect Module) compared to attention-based training (Presence Module), or no training. Additionally, 6-month attention-based and socio-cognitive training (Presence and Perspective Modules) lead to decreased overall oxytocin levels compared to three months of Presence training alone. Contrary to our hypothesis of oxytocin acting as a stress buffer, training-induced changes in overall oxytocin release were unrelated to training-induced reductions in cortisol and subjective stress reactivity. In our second sub-study, and again in contrast to our a priori hypothesis, LKM did not influence acute oxytocin release compared to rest. Similar to sub-study 1, there was an influence of the preceding 3-month, socio-affective Affect Module on *overall* oxytocin release. However, overall oxytocin levels after Affect training were increased relative to no training.

Regarding our first hypothesis (i.e., elevated overall and stress-induced plasma oxytocin levels specifically after the compassion-based Affect Module), we can infer from the detected oxytocin pattern, that distinct types of mental training had differential effects on overall oxytocin release before, during and immediately after psychosocial stress. Other than expected, however, overall oxytocin levels were lowest after 3-month Affect training, and highest after 3-month Presence training. After 6-month Presence and subsequent Affect training, or Presence and subsequent Perspective training, overall oxytocin levels ranged in the middle. Possibly, due to an initial rise in hormone levels after the Presence Module, a drop in overall oxytocin, as observed after the Affect Module, was delayed. When comparing stress and LKM/rest sessions, participants of the Affect Module exhibited similar overall oxytocin release during psychosocial stress and LKM. In contrast, participants without prior training revealed considerably lower overall oxytocin levels during rest, compared to both, their own levels during psychosocial stress, and Affect participants during LKM. Thus, although the overall oxytocin results during LKM/rest correspond to our initial expectation of increased oxytocin release after the training of socio-affective skills, the inverse pattern during psychosocial stress proposes a more complicated mechanism than a simple training-induced rise in oxytocin availability.

We suggest that our unexpected results are best understood against the backdrop of Quintana and Guastella's (2020) theory of oxytocin as an allostatic hormone. The authors propose that oxytocin facilitates stability of the organism through changing environments in order to promote survival. The term allostasis, in contrast to homeostasis, explicitly includes the ability to anticipate future changes, and to adjust physiological set points to enable better coping with this change (Quintana and Guastella, 2020). It could thus be argued that, to ensure the organism's stability, oxytocin is released whenever individuals anticipate events that feel relevant for survival, no matter whether positive or negative in valence. Regarding

our stress test, we accordingly suggest that the Affect Module reduced overall oxytocin levels compared to no training because the anticipated emotional relevance of a psychosocial challenge like the TSST was lowered by the three months of socio-affective training. Thus, by learning to accept difficult emotions, and generate positive affect towards oneself and others, the TSST may have lost its emotional saliency and threatening character for Affect-trained participants. Regarding LKM/rest, anticipating to meditate would have gained higher emotional relevance for Affect-trained participants than anticipating to rest for no training participants. Clearly, having practiced LKM almost daily for three months would increase the salience, and hence the anticipated emotional relevance of attending the meditation session.

Our next hypothesis suggested that training-induced changes in oxytocin release would mediate training-induced reductions in subjective and cortisol stress reactivity during psychosocial stress. Interestingly, the overall oxytocin pattern during psychosocial stress partially mirrored that of cortisol reactivity, with relatively higher stress-induced cortisol levels in the Presence as opposed to Affect and Perspective Modules (Engert et al., 2017). This means that especially socio-affective and socio-cognitive mental training reduced cortisol secretion after psychosocial stress. Yet, we found no association between training-induced changes in overall oxytocin release, and changes in self-reported or cortisol stress reactivity. We accordingly suggest that reduced stress reactivity after mental training is not driven by changes in peripheral oxytocin availability, or vice versa. Although a stress-buffering effect of oxytocin on cortisol release is a frequent finding in the animal literature (Jurek and Neumann, 2018), and in human endogenous oxytocin or stimulation studies (e.g. Heinrichs et al., 2003, 2001; Kubzansky et al., 2012; Light et al., 2000; Quirin et al., 2011), the present lack of an association is not overly surprising. First, because typically, reactive rather than overall oxytocin levels are considered, and second, because the examination of peripheral oxytocin in humans provides a somewhat inconsistent picture. In fact, stress-

induced peripheral oxytocin release was mostly shown to correlate *positively* with cortisol release (Bernhard et al., 2018; de Jong et al., 2015; Engert et al., 2016). Only one study found an inverse relationship between average plasma oxytocin and saliva cortisol levels during psychosocial stress (Pierrehumbert et al., 2010). Thus, our results support the notion that peripheral oxytocin levels do not mirror the stress-buffering role of the neuropeptide, as found with central oxytocin release or in stimulation studies.

Our data confirm that plasma oxytocin is sensitive to acute psychosocial stress. Independent of mental training, it responded to the stressor with a post-stressor peak and a drop back to baseline levels within one hour after stress, as shown by previous research (e.g. Bernhard et al., 2018; de Jong et al., 2015; Engert et al., 2016; Light et al., 2000; Pierrehumbert et al., 2010). Contrasting the findings by Bellosta-Batalla et al. (2020a), oxytocin levels did not rise in response to one LKM session. While the practiced meditation technique (loving-kindness vs. mindfulness) might play a role in this inconsistency, it is also possible that a single meditation session is not a strong enough stimulus to reliably trigger acute oxytocin release.

The meditation session yielded additional insights into the nature of LKM. At baseline, participants of the 3-month Affect Module exhibited decreased cortisol levels, and less positive valence compared to participants without prior training. Contrarily, emotional warmth and subjective stress were elevated. These baseline differences may originate from the fact that Affect-trained participants, knowing they would be practicing meditation, anticipated a more demanding task than no training participants. Importantly, engaging in meditation increased emotional warmth and positive valence, despite being rated as more effortful than rest. The latter results closely reflect previous reports from the ReSource Project. Przyrembel et al. (2019) found that the qualitative experience of LKM is associated with feelings of love, warmth, and sensations around the heart. Lumma et al. (2015), showed

that LKM is not always perceived as relaxing. Rather, it seems to require effort without being stressful.

There are several limitations to the current study. First, there is no agreement on a standard protocol for oxytocin antibody and assay methods. Importantly, however, the utilized radioimmunoassay has been standardized and validated in numerous animal and human studies (Landgraf and Neumann, 2004). Second, due to our specific a-priori focus on the influence of LKM on oxytocin availability, sub-study 2 was realized only in the context of the Affect Module. Given that stress reduction was found equally after Affect and Perspective training (Engert et al., 2017), it would have been informative to also shed light on oxytocin release during Observing-thought Meditation. Third, due to multicollinearity, sex and hormonal status could only be considered as covariates in all statistical analyses. Examining interactions of sex and hormonal status with group/cohort or time slopes was not possible. Fourth, oxytocin was assessed only on the day of testing. The sample taken immediately prior to stress and LKM, however, is rather an estimate of anticipation, than an actual baseline. Fifth, every participant consumed a snack upon arrival to equalize the influence of unsystematic food intake on blood sugar levels. Because food intake affects oxytocin release (Aulinas et al., 2019; Jurek and Neumann, 2018), however, this may have affected our results. Sixth, due to limited availability of antibodies and low intra-assay variability (of <10%), oxytocin samples were not assayed in duplicate. Lastly, we calculated post-hoc power analyses to give a rough estimate for the power of our effects. These post-hoc power analyses need to be interpreted with caution. As power analyses for multi-level models are still a developing topic with many unresolved problems (Kumle et al., 2021), we were not able to conduct post-hoc power analyses for the reported Omnibus F tests of the LMM including post-hoc comparisons. Instead, in the Supplemental Material (Table S5), we show post-hoc power analyses for a LMM with regression slopes and t-tests. Further, several authors

recommend to not conduct power analyses post-hoc (Hoenig and Heisey, 2001; Levine and Ensom, 2001; Zhang et al., 2019), but instead report confidence intervals for all significant effects (Hoenig and Heisey, 2001; Yuan and Maxwell, 2005). Future research is encouraged to include a priori power calculations when replicating our findings.

In summary, we found that contemplative mental training modified overall plasma oxytocin levels, both during a psychosocial stress paradigm, and during a short compassion-based mental practice session. After 3-month socio-affective mental training, participants exhibited similar oxytocin release in both situations, stress and LKM. Without prior training, participants revealed higher overall oxytocin levels in response to stress than rest. Training-induced changes in oxytocin release were not associated with the stress-buffering effect of contemplative mental practice. Based on Quintana and Guastella's (2020) theory of oxytocin as an allostatic hormone, we suggest that changes in oxytocin release are due to changes in the anticipated emotional relevance of specific events: A psychosocial challenge situation may have lost its emotional saliency after three months of socio-affective training focusing on the acceptance of difficult emotions and generation of positive affect. In contrast, LKM practice may have become a more emotionally salient stimulus over time. Overall, our data suggest that stress reduction after compassion-based mental training develops independently of peripheral oxytocin availability. They further highlight the notion that oxytocin is not solely an anxiolytic or affiliation-boosting hormone. In line with Quintana and Guastella's theory, its role rather seems to be that of an allostatic hormone, reacting to emotionally salient events that are deemed relevant for survival. Accordingly, oxytocin seems to be implicated in diverse processes and behaviours, aiming to facilitate stability in changing environments.

5 Declarations

Acknowledgements: We are thankful to the members of the Social Neuroscience Department involved in the *ReSource Project* over many years, in particular to Astrid Ackermann,

Christina Bochow, Matthias Bolz and Sandra Zurborg for managing the large-scale longitudinal study, to Elisabeth Murzik, Nadine Otto, Sylvia Tydecks, and Kerstin Träger for help with recruiting and data archiving, to Henrik Grunert for technical assistance, and to Hannes Niederhausen and Torsten Kästner for data management. Thank you also to the research assistants and students, especially Anna Koester, whose help with data collection was indispensable.

Declarations of interests: none.

Funding: This study forms part of the ReSource Project headed by T.S. Data for this project were collected between 2013 and 2016 at the former Department of Social Neuroscience at the MPI CBS Leipzig. T.S. (Principal Investigator) received funding for the ReSource Project from the European Research Council (ERC) under the European Community's Seventh Framework Program (FP7/2007–2013) ERC grant agreement number 205557. The funding organizations had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

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Author Statement

Author contributions: T.S. initiated and developed the ReSource Project and secured all funding. T.S., P.V., and V.E. designed the experiment. P.V. and V.E. were involved in the data collection; K.H., P.V., and V.E. analyzed the data. K.H. and V.E. drafted, and all authors critically revised the manuscript.

Declaration of Interests

Declarations of interests: none.

Figures

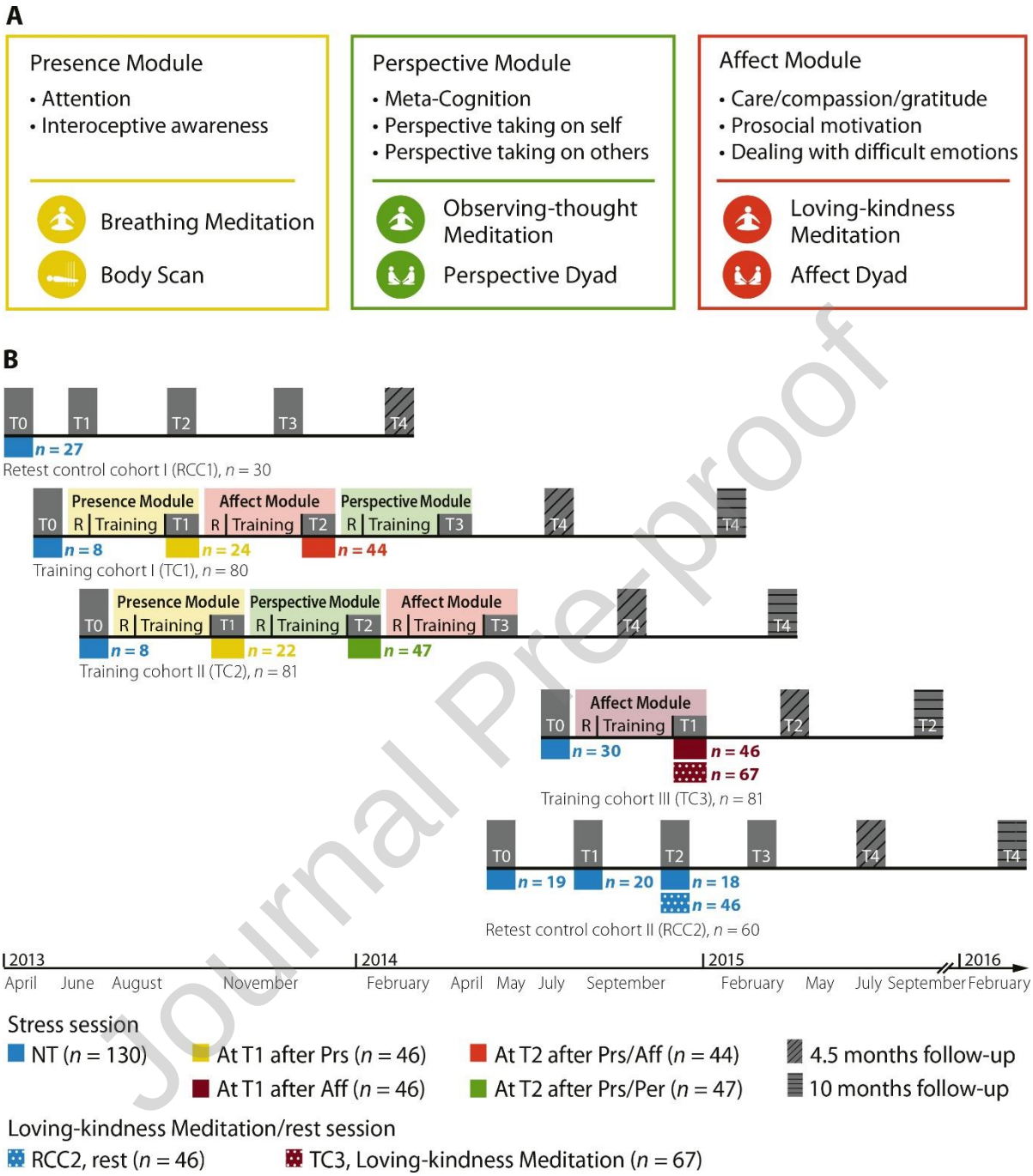

Figure 1. Methodological details of the ReSource Project. (A) Training modules and core exercises of the ReSource Project. (B) Time points of stress and Loving-kindness Meditation/rest testing within the greater context of the ReSource training timeline, and cohort membership of tested participants. NT = no training; Prs = Presence; Aff = Affect; Prs/Aff = Presence/Affect; Prs/Per = Presence/Perspective.

Stress session: 313 out of initial 332 ReSource participants (dropout: 19);

Loving-kindness Meditation/rest session: 113 out of initial 141 participants from TC3 and RCC2 (dropout: 28).

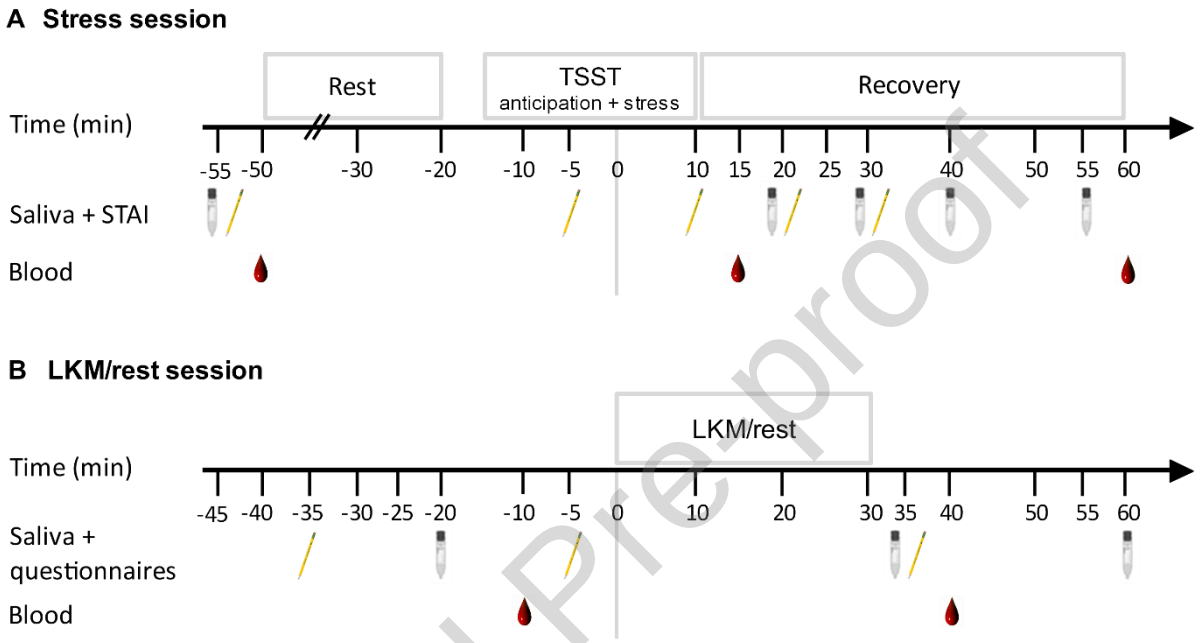

Figure 2. Testing timeline. (A) Time is coded in minutes before and after stressor onset at 0 min. Cortisol was assessed via saliva samples, oxytocin via blood samples, and subjective stress via the state scale of the State Trait Anxiety Inventory (STAI). (B) Time is coded in minutes before and after Loving-kindness Meditation/rest onset at 0 min. Cortisol was assessed via saliva samples, oxytocin via blood samples, and subjective stress via a visual-analogue rating scale. Emotional warmth was only assessed at -35 min and 35 min, and subjective effort at 35 min using visual-analogue rating scales. Valence and arousal were assessed using the Affect Grid.

LKM: Loving-kindness Meditation.

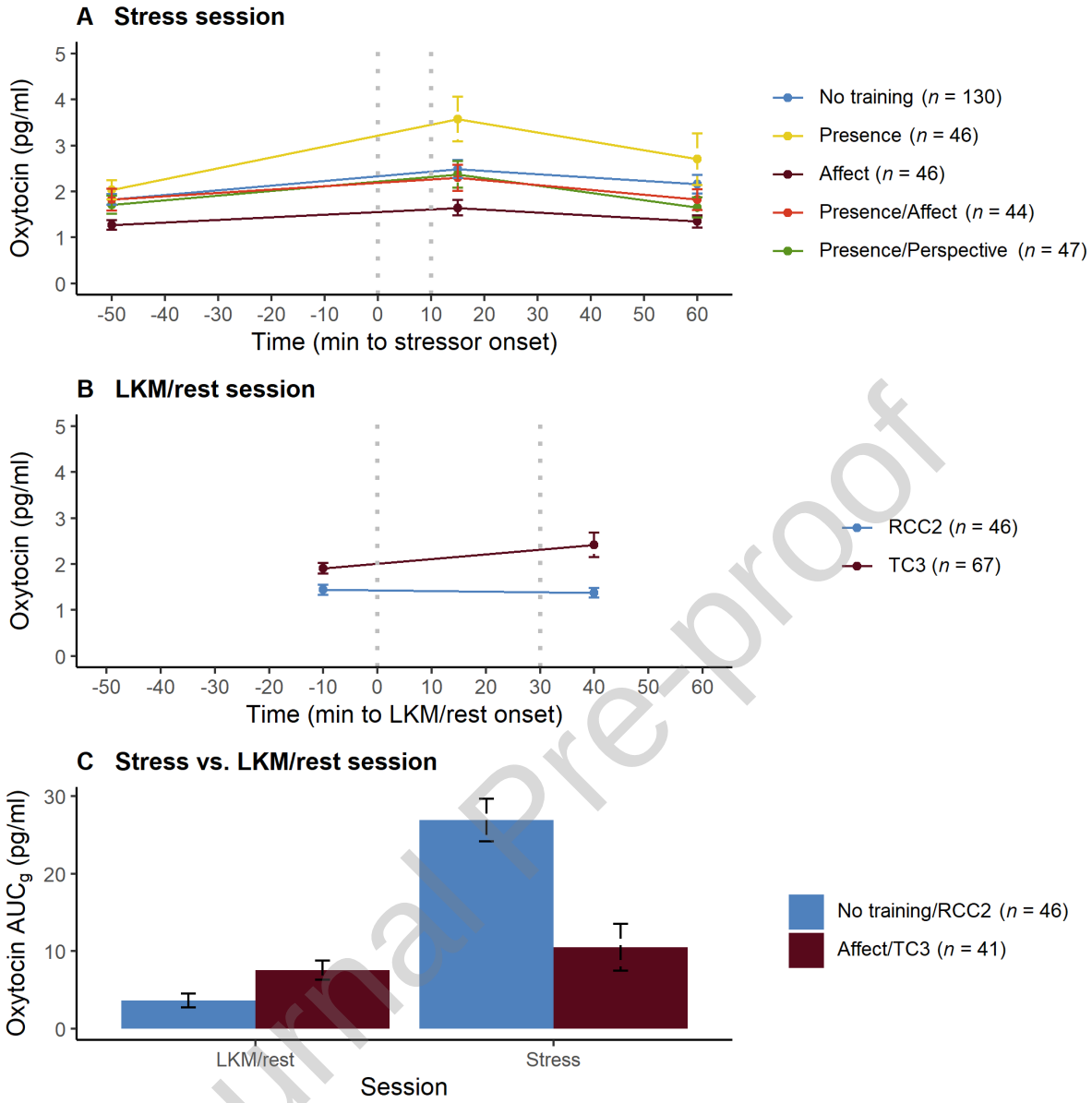

Figure 3. (A) Means of plasma oxytocin levels (raw data) in the different mental training groups during psychosocial stress. Error bars represent the standard errors of the mean. (B) Means of plasma oxytocin levels (raw data) during Loving-kindness Meditation (TC3) or rest (RCC2). Error bars represent the standard errors of the mean. (C) Means of plasma overall oxytocin levels (AUC_g) from both, stress and Loving-kindness Meditation/rest session. Error bars represent the standard errors of the mean. Oxytocin AUC_g was corrected for the number of included oxytocin measurements.

Because covariates are not considered, results may deviate from the model-derived depiction.

LKM: Loving-kindness Meditation.

Table 1. (A) Omnibus *F* tests in a LMM examining training effects on oxytocin plasma levels during psychosocial stress. (B) Omnibus *F* tests in a LMM examining the effect of a single Loving-kindness Meditation versus rest on oxytocin plasma levels. (C) Pairwise Tukey-Kramer post-hoc comparisons and Cohen's *d* for group effects on oxytocin plasma levels during psychosocial stress.

Aff, Affect; NT, no training; Prs, Presence; Per, Perspective.

Fixed effects	**A**		**B**	
	F (df)	*p*	*F* (df)	*p*
Intercept (peak or baseline)	233.80 (406)	< .001	21.97 (125)	< .001
Reactivity slope/time	69.38 (553)	< .001	0.13 (105)	.718
Recovery slope	60.26 (553)	< .001		
Group/cohort	6.35 (444)	< .001	6.62 (164)	< .05
Reactivity slope/time* group/cohort	1.51 (553)	.198	1.26 (105)	.264
Recovery slope* group/cohort	2.03 (553)	.088		
Age	2.06 (279)	.152	2.52 (100)	.115
Sex/hormones	6.36 (280)	< .001	1.31 (100)	.276
Daytime			0.15 (100)	.701
Random effects				
	Variance *(SD)*		Variance *(SD)*	

Individual	0.276 (0.525)	0.127 (0.357)

C

	Prs		**Aff**		**Prs/Aff**		**Prs/Per**	
	t (df)	*d (95% CI)*	*t* (df)	*d (95% CI)*	*t* (df)	*d (95% CI)*	*t* (df)	*d (95% CI)*
NT	-2.13 (279)	-0.38 [-0.73; -0.03]	3.16* (282)	0.56 [0.21; 0.92]	0.69 (278)	0.12 [-0.23; 0.48]	1.57 (277)	0.28 [-0.07; 0.64]
Prs			4.40*** (281)	0.95 [0.50; 1.39]	2.34 (278)	0.51 [0.07; 0.94]	3.07* (278)	0.66 [0.23; 1.10]
Aff					-2.05 (280)	-0.44 [-0.87; -0.01]	-1.30 (280)	-0.28 [-0.71; 0.15]
Prs/Aff							0.74 (277)	0.16 [-0.27; 0.59]

Satterthwaite approximation for degrees of freedom. Models based on 849 observations from 288 participants (A + C, stress session) and 214 observations from 107 participants (B, LKM/rest session).

† $p < .10$, * $p < .05$, ** $p < .01$, *** $p < .001$

Table 2. Omnibus *F* tests in ANCOVAs examining associations between overall plasma oxytocin levels (AUC_g) and stress markers (cortisol reactivity, STAI reactivity) during psychosocial stress.

	Cortisol reactivity		**STAI reactivity**	
	F (df)	*p*	*F* (df)	*p*

Intercept	0.02 (258)	.875	293.22 (260)	< .001
Oxytocin AUC_g	0.11 (258)	.745	2.24 (260)	.136
Group	4.75 (258)	< .01	5.92 (260)	< .001
Oxytocin AUC_g*group	1.82 (258)	.125	0.24 (260)	.913
Age	0.62 (258)	.432	0.04 (260)	.846
Sex/hormones	8.75 (258)	< .001	0.10 (260)	.752
Daytime	6.50 (258)	< .05		

Models based on 273 participants for cortisol and 272 participants for STAI. Oxytocin AUC_g = overall oxytocin plasma levels.

Table 3. Omnibus *F* test in LMMs and one ANCOVA (effort) examining effects of one Loving-kindness Meditation on levels of cortisol and subjective experience (stress, warmth, valence, arousal and effort).

	Cortisol		**Subjective stress**		**Warmth**	
Fixed effects						
	F (df)	*p*	*F* (df)	*p*	*F* (df)	*p*
Intercept	208.54 (124)	< .001	246.58 (136)	< .001	282.57 (139)	< .001
Time	11.71 (210)	< .001	102.52 (218)	< .001	3.13 (105)	.080
Cohort	10.66 (162)	< .01	7.71 (178)	< .01	12.59 (174)	< .001
Time*cohort	3.70 (210)	.056	0.42 (218)	.519	13.44 (105)	< .001
Age	0.12 (99)	.734	2.89 (106)	.092	1.63 (107)	.205
Sex/	1.81 (99)	.151	0.60 (107)	.441	0.00 (106)	.976

hormones						
Daytime	112.83 (99)	< .001	1.18 (107)	.279	0.18 (106)	.674

Random effects

	Variance *(SD)*	Variance *(SD)*	Variance *(SD)*
Individual	0.247 (0.497)	1.889 (1.374)	1.801 (1.342)

	Valence		**Arousal**		**Effort**	
Fixed effects						
	F (df)	*p*	*F* (df)	*p*	*F* (df)	*p*
Intercept	947.12 (140)	< .001	764.55 (162)	< .001	28.41 (101)	< .001
Time	21.29 (220)	< .001	55.33 (220)	< .001	-	-
Cohort	7.84 (186)	< .01	2.49 (232)	.116	36.54 (101)	< .001
Time*cohort	4.44 (220)	< .05	3.08 (220)	.081	-	-
Age	0.62 (107)	.432	0.00 (107)	.985	0.00 (101)	.957
Sex/ hormones	1.79 (107)	.184	0.53 (107)	.468	2.31 (101)	.132
Daytime	0.33 (107)	.567	9.67 (107)	< .01	0.92 (101)	.339
Random effects						
	Variance *(SD)*		Variance *(SD)*		Variance *(SD)*	
Individual	1.081 (1.040)		0.904 (0.951)		-	

Satterthwaite approximation for degrees of freedom. Models based on 318 observations from 106 participants (cortisol), 331 observations from 112 participants (stress), 334 observations from 112 participants (valence, arousal), 215 observations from 112 participants (warmth), and 106 participants (effort).

Highlights

- Independent of mental training, oxytocin reacted to stress but was unaffected by Loving-kindness Meditation
- Compassion-based mental training influenced overall oxytocin release during stress and meditation
- Training-induced changes in overall oxytocin release did not mediate stress reduction after mental training