**Supplementary Material**

**Effects of Mindfulness-Based Stress-Prevention**

**on Serotonin Transporter Gene Methylation**

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**Supplementary Materials and Methods**

**Participants**

Participants were recruited at the Medical School of Heidelberg University, Germany. All participants were medical students enrolled in their third semester heading towards their first important exam at the end of the term. Exclusion criteria were chronic severe somatic diseases (e.g., cancer, acute hepatitis etc.); chronic severe psychiatric disorders (e.g., schizophrenia, neurodevelopmental disorders, substance abuse or dependence etc.); heavy smoking (>20 cigarettes per day); the intake of psychiatric medication; and pregnancy. Participants were recruited via e-mail. All potential participants completed a questionnaire assessing eligibility at an information session before the start of the study. One participant from the MBI group was excluded from all analyses because of self-reported intake of psychiatric medication throughout the intervention. Sociodemographic (e.g., age) and health-related (e.g., smoking behavior) sample characteristics were assessed using a detailed self-report checklist. Participants received monetary compensation upon study completion. One participant from each group left the study by post-intervention for personal reasons.

**Intervention**

The MBI offered in this study was embedded in a standard course curriculum for medical students in the preclinical phase of Medical School at Heidelberg University. The focus was on guided exercises like mindful breathing meditation, a relaxation exercise and a body scan in the supine position. Each session started with breathing meditation, continued with the theoretical model of mindfulness- and stress-related topics followed by discussions including a group-based inquiry on personal experiences, and closed with a practical contemplative part applying progressive muscle relaxation and a body scan. At the one-day introduction session, all participants received audio files of the stress-preventive techniques to facilitate home-practice. However, participants were not required to exercise stress-prevention practices at home, but they could choose to do so voluntarily. Despite the positive influence of daily meditation practice on health parameters, current studies suggest great heterogeneity in its implementation and adherence [S1].

**Assessment of control variables**

As control variables, we assessed factors which were previously shown to be linked with *SLC6A4 DNAm* (see Palma-Gudiel and colleagues [S2]). Thus, chronic stress was assessed using the 12-item screening scale of the Trier Inventory for the Assessment of Chronic Stress (TICS) [S3]; childhood adversities were assessed using the short version of the Childhood Trauma Questionnaire (CTQ) [S4, S5]; and recent life events were assessed using a one-item assessment that asked about severe changes of life circumstances within the last year (no/yes). At post-intervention, a retrospective questionnaire assessed compliance with the MBI by asking whether participants practiced mindfulness exercises learned from the MBI in their everyday life (no/yes). In addition, participants were asked whether they felt like being able to cope better with stress compared to before the intervention (no/yes). Visual analogue scales were used to rate the feasibility of the mindfulness practices (0 = not at all, 10 = very good) and the extent to which MBI participants felt a reduction of stress (0 = not at all, 10 = very high).

**Sampling of DNA and mRNA**

Peripheral blood was drawn into EDTA coated tubes. PAXgene blood RNA Tubes (PreAnalytiX, Qiagen, Hilden, Germany) were collected for the assessment of mRNA.

**Genotyping**

Genotyping was conducted by Varionostic GmbH (Ulm, Germany) using standard procedures. The gDNA was isolated with the ReliaPrep Blood gDNA Miniprep System (Promega, Madioson, WI, USA) according to the manufacturer’s protocol for peripheral blood collected in EDTA coated tubes. PCR was performed in 10µl reactions with the OneTaq DNA polymerase (New England Biolabs, Ipswich, MA, USA) according to the manufacturer’s protocol in a peqSTAR thermocycler (peqLab, Erlangen, Germany) in 3 steps: (1) denaturation at 94° C for 30 seconds, (2) 32 cycles repeating denaturation at 94° C for 20 seconds, annealing at 60° C for 30 seconds and extension at 68° C for 1 minute, (3) final extension at 68° for 5 minutes. The PCR-product was digested with the fast digest Hpa II (Thermo Fisher Scientific, Darmstadt, Germany) according to the manufacturer’s protocol and analyzed via gel electrophoresis (2% agarose-gel in TAE, visualized in gel red). Primer names and sequences [S6] are depicted in Table S1. While considering LG as being functionally similar to the S allele [S7], genotypes were classified as follows: LL (LALA), LS (LASA, LALG), SS (SASA, LGLG, SALG). Because of the relatively small sample size, we used only the biallelic classification of the *5-HTTLPR/rs25531* mini haplotype for statistical models.

**Quantitative Real-Time Reverse Transcriptase PCR**

For *SLC6A4* mRNA expression analyses, PAXgene blood RNA Tubes (PreAnalytiX) were sent to Varionostic GmbH, where the quantitative real-time PCR was conducted. *ACTB* (beta-actin) and *GAPDH* (Glyceraldehyde-3-phosphate dehydrogenase) were used as reference genes. To quantify gene expression of *SLC6A4* and of reference genes, RNA was isolated with the GeneJET Stabilized and Fresh Whole Blood RNA Kit (Thermo Fisher Scientific, Darmstadt, Germany) according to the manufacturer’s protocol for PAXgene blood RNA tubes (PreAnalytiX, Qiagen, Hilden, Germany). An additional DNase I step according to the manufacturer’s protocol was performed with the DNase I (Thermo Fisher Scientific, Darmstadt, Germany). RNA was reverse transcribed to cDNA with the High Capacity RNA-to-cDNA Kit (Thermo Fisher Scientific, Darmstadt, Germany) for 60 minutes at 37° C. PCR was conducted in 2 steps: (1) denaturation at 95° C for 2 minutes, (2) 45 cycles repeating denaturation at 95° C for 5 seconds, annealing at 60° C for 10 seconds and extension at 72° C for 20 seconds. Expression of *SLC6A4*, *GAPDH* and *ACTB* was measured on the Rotorgene Q (Qiagen, Hilden, Germany) with the SensiFAST SYBR No-ROX Kit from Bioline (Luckenwald, Germany). Table S2 contains the names and sequences of the primers [S8] used for expression analyses of *SLC6A4*, *GAPDH* and *ACTB*. An initial quality control of CT values identified *GAPDH* as almost zero expressed in most of the samples (CT values: mean = 29.3, SD = 8.29), indicating an absence of *GAPDH* mRNA; this could be a result of spurious amplification [S9]. As a consequence, *GAPDH* was excluded from subsequent analyses. Relative quantification of *SLC6A4* expression was calculated using the 2-ΔΔCT – Method [S10] (ΔΔCT = (CT, *SLC6A4*– CT, *ACTB*) post-intervention – (CT, *SLC6A4*– CT, *ACTB*) pre-intervention).{Schmittgen, 2008 #45} Fold change values < -1 represent a decrease in *SLC6A4* expression on the same scale as increases in *SLC6A4* expression with values > 1. In summary, these values are a measure of the intra-individual change in *SLC6A4* expression from pre- to post-intervention, normalized to *ACTB* and relative to *SLC6A4* expression at pre-intervention.

**Methylation analyses**

Samples of peripheral blood drawn in EDTA coated tubes were provided to Varionostic GmbH, who conducted the methylation analyses by bisulfite pyrosequencing. Percentage methylation was quantified within a promoter-associated 799-bp CpG island of *SLC6A4* as first outlined by Philibert and colleagues [S11] and described in detail elsewhere [S8] The assay used for methylation analyses was the same as reported in previous studies [S8, S12]. However, because of limited resources, we were not able to cover the biochemical analyses of the whole CpG island, as had been done in these previous studies. Therefore, from the biochemical assay [S8, S12], three sequences comprising CpG sites 1-7, CpG sites 23-32 and CpG sites 33-42 were not analyzed. We chose to analyze CpG sites in which we could expect sufficient variability of methylation values, as seen in previous studies which used the exact same assay for the quantification of *SLC6A4 DNAm* in a comparable group of participants [S8, S12]. Furthermore, we made sure that the CpG sites had already been reported in other publications on a topic with immediate relevance for our study, namely reports on the association of *SLC6A4 DNAm* with recent life stress (van der Knaap et al. [S13], CpG sites 43-83), with environmental stress (Alasaari et al. [S14]; CpG sites 8-12 and Lei et al. [S15]; CpG sites 8, 9, 12, 13, 55, 76), or chronic stress (Duman and Canli [S16]; CpG sites 13-83). Of note, in the latter manuscript, associations of *SLC6A4 DNAm* with chronic stress were mainly driven by a factor comprising CpG units located at the start of the CpG island, thereby adding to the assumed relevance of this specific region for stress reactivity. This led us to focus on CpG sites 8 to 22 as well as on 43 to 83. Consequently, in total, 56 CpG sites within the 799-bp CpG island were analyzed by bisulfite pyrosequencing. The analyzed base sequence and the positions of the CpG sites are illustrated in Fig. S1. Total methylation levels for each CpG site are depicted in Fig. S2. Nucleotide positions are according to NCBI genome browser sequence NG\_011747.2. To conduct CpG site methylation analyses, the gDNA was bisulfate treated using the EZ DNA Methylation Gold Kit (Zymo Research, Range, CA, USA). For PCR, 3 pairs of primers were used to generate 3 amplicons from the bisulfite-converted DNA (bisDNA). As polymerase, the HotStarTaq polymerase (Qiagen, Hilden, Germany) was used. PCR was performed with the following steps for amplicon 1 and 2: (1) denaturation at 95° C for 15 minutes, (2) 49 cycles repeating denaturation at 95° C for 35 seconds, annealing at 57° C for 35 seconds and extension at 72° C for 35 seconds, (3) final extension at 72° C for 5 minutes. For amplicon 3, the annealing temperature in step 2 was set to 52° C. All other conditions of the PCR were identical for the 3 amplicons. As controls, methylated and unmethylated control DNA samples were used for the bisulfite conversion reaction, the extension, and the pyrosequencing reaction (EpigenDx Inc., MA, USA). For pyrosequencing analyses, the samples were prepared according to standard procedures using Vacuum Prep Tool. First, 12-15 μl PCR product was immobilized to 2 μl Streptavidin SepharoseTM HP beads (GE Healthcare, Piscataway, NJ, USA). In a second step it was annealed to 0.8-1.0 μl of the sequencing primer (5 μM) for 2 minutes at 80° C. CpG analyses and subsequent quality check were done with PyroMark Q24 software. More than 98% of all measured methylation values passed quality control. Amplicon and sequencing primers are shown in Table S3. In addition, to enhance the comparability to future studies on changes in *SLC6A4 DNAm* following psychological interventions, Table S4 depicts which of the CpG sites pyrosequenced in our study are also covered by frequently used microarrays for measuring DNA methylation.

**Statistical analyses**

Data preparation and statistical analyses were conducted using R 3.4.1 [S17]. Two-level multilevel models (MLMs; observation nested in participants) were fitted with the function *lme* of the *nlme-*package [S18] using the Restricted Maximum Likelihood estimation method. Continuous predictors were grand mean centered while categorical variables were dummy-coded. Random intercepts were allowed for each participant in all MLMs. Heteroscedasticity of model residuals was identified by plotting residuals against values of variance covariates [S19] and modeled by using the “exponential of covariate” function of the *nlme*-package [S18-S20]. Subsequently, improvements in model fit were formally tested by conducting likelihood-ratio tests for nested models as well as by interpreting the Akaikes information criterion [S19, S20]. In a last step,the validity of distributional assumptions was assessed visually for each final model [S19]. The significance level was set to *p* ≤ 0.05. Bonferroni corrections were applied to CpG site-specific analyses (*N* = 56) to account for multiple testing; thus, setting the significance threshold to *p* < 0.0009.

Changes in mindfulness were analyzed via a MLM with a group-(0 = control group, 1 = MBI group)-by-time (0 = pre-intervention, 1 = post-intervention) interaction, controlling for sex (0 = men, 1 = women) and age. To test the hypothesis that group-specific changes in *SLC6A4 DNAm* occur over the course of the intervention, we analyzed group-by-time interactions using a MLM. Mean *SLC6A4 DNAm* across all analyzed CpG sites (*n* = 56) was calculated and predicted by this interaction term controlling for sex, age, BMI, mindfulness, childhood adversities, recent life events (0 = no, 1 = yes), chronic stress, intake of hormonal contraceptives (0 = no, 1 = yes), smoking status (0 = no, 1 = yes) and the *5-HTTLPR/rs25531* mini haplotype. Additional MLMs were fitted the same way to compare changes in *SLC6A4 DNAm* between groups at each individual CpG site. Furthermore, sensitivity analyses were conducted in order to ensure the stability of all results pertaining to group-specific changes in *SLC6A4 DNAm*. First, final MLMs were re-estimated while including baseline *SLC6A4 DNAm* as additional predictor. Second, participants who self-reported psychiatric diagnosis were excluded from the analysis.

Self-report data on feasibility and stress reduction pertaining to the MBI were related to change scores of mean *SLC6A4 DNAm* (percentage point change; methylation post-intervention – methylation pre-intervention) via Pearson’s product-moment correlation. Furthermore, subgroups were formed according to compliance to home practice of mindfulness exercises (no/yes) and according to self-reported improvements in stress-coping (no/yes) following the MBI. Change scores of mean *SLC6A4 DNAm* between these subgroups were compared via Welch’s t-tests (two-sided) with Cohen’s *d* as an effect size measure [S21]. In this vein, we conducted exploratory correlational analyses to examine a possible association of change scores of mean *SLC6A4 DNAm* with change scores of mindfulness (mindfulness post intervention – mindfulness pre-intervention).

To analyze dynamics in *SLC6A4* expression changes over the course of the study, fold change values of *SLC6A4* expression were compared between groups using a Welch’s t-test as described above. Additionally, linear regression predicted *SLC6A4* expression fold changes by group and *5-HTTLPR/rs25531* to control for a possible impact of polymorphic variations on group-specific changes in *SLC6A4* expression. Furthermore, fold change values of *SLC6A4* expression were associated with change scores of mean *SLC6A4 DNAm* via Pearson’s product-moment correlations.

**Supplementary Results**

**Sample characteristics**

Table S5 shows sample characteristics by study group. At baseline, the groups only differed with regard to body mass index but not with regard to age, smoking, sex, hormonal contraceptive use, recent life events, childhood adversities, chronic stress, mindfulness and mean *SLC6A4 DNAm* (Table S5). Allele frequencies of *5-HTTLPR/rs25531* did not significantly differ from the Hardy-Weinberg equilibrium in our sample (*χ²*(1) = 0.4994, p = 0.4797). The MBI group and the control group did not differ in genotype group distribution (*χ²*(2) = 3.9303, *p* = 0.1401). Self-report data on compliance, feasibility, stress-coping and stress reduction from the MBI group can be found in Table S6.

**Associations of control variables with mean *SLC6A4 DNAm* and sensitivity analyses**

Among all control variables, we found sex (*b* = 0.7543, SE= 0.2272, *p* = 0.0015) and age (*b* = 0.0898, SE= 0.0437, *p* = 0.0443) to be positively associated with mean *SLC6A4 DNAm*. This indicates higher mean *SLC6A4 DNAm* for women and older participants. Notably, neither *5-HTTLPR/rs25531* nor any of the other control variables were associated with mean *SLC6A4 DNAm*. Sensitivity analysis showed that group-specific changes in mean *SLC6A4 DNAm* remained significant when entering baseline *SLC6A4 DNAm* as additional predictor and when excluding participants who self-reported psychiatric diagnoses.

**Changes in CpG site-specific methylation****following the MBI**

In general, a constant pattern of group-specific methylation changes was found at CpG sites located in the *SLC6A4* promoter region and in parts of the first exon. These differential changes were significant for 13 CpG sites (8, 10, 11 and 13 – 22). Methylation decreased in the MBI group while it increased in the control group in 10 out of these 13 CpG sites (11 and 14 – 22). Of all CpG sites located downstream of CpG site 22, only three (48, 53 and 65) showed significant group-specific changes. Significant results were unaffected by baseline methylation and self-reported psychiatric diagnoses. Detailed results, including group-specific methylation percentage point changes, can be obtained from Table S7. For illustration purposes, we present CpG site-specific methylation changes which did not significantly differ between groups in Fig. S3 (for all other CpG sites, see Fig. 1 in the research letter). Of note, we found sex differences in methylation at 14 CpG sites (62, 69, 71-79, 81-83) located in close proximity to the north shore of the CpG island (Table S8). Methylation at all 14 CpG sites was positively associated with sex (women showing a higher methylation in comparison to men), which is in line with previous studies [S22, S23].

**Changes in *SLC6A4* expression in association with changes in CpG site-specific methylation**

Additional exploratory analyses revealed associations of changes in CpG site-specific methylation with changes in *SLC6A4* expression for 15 CpG sites (see Table S9 for detailed results). In summary, despite not surviving corrections for multiple testing, almost all associations of changes in CpG site-specific methylation with changes in *SLC6A4* expression were negative.

**Supplementary Discussion**

In-depth CpG site specific analyses revealed that differential changes in methylation levels were most pronounced at CpG sites located near the TSS in thepromoter region and in the first exon. The corresponding analyses showed a pattern of decreasing methylation in the MBI group and increasing methylation in the control group. When considering high levels of *SLC6A4 DNAm* as related to stress as described above, the workload and stress because of the important upcoming exam might have triggered the increasing methylation at CpG sites close to the TSS in the control group. In comparison, participants from the intervention group showed a decrease in methylation levels at these CpG sites while still being exposed to the same stressful demands. This leads to assume that changes in *SLC6A4 DNAm* in response to stressor exposure could have been reversed by the MBI. Only recently, Mc Ewen [S24] proposed that experience-driven changes in the epigenetic signature can be “redirected” through behavioral interventions to achieve “a successful outcome in the face of adversity” (p. 2). Our results do provide scarce evidence for this assumption.

However, despite changes *SLC6A4 DNAm* were associated with changes in parameters on stress, they were not associated with changes in mindfulness. This indicates that the stress-preventive effects of the MBI, as measured via a decreasing *SLC6A4 DNAm*, cannot only be explained by changes in mindfulness. Rather, it’s highly possible that other aspects of the MBI, which exerted an effect on stress, were driving this change in *SLC6A4 DNAm*. These include the relaxation exercises, the group-based inquires on personal experiences, the theoretical knowledge on stress and its effects on the body, and interactions among them or with mindfulness. Therefore, further research is needed to disentangle the exact mechanism – or the interactions of mechanisms – which define the stress-preventive effects of mindfulness-based stress-prevention on changes in *SLC6A4 DNAm.* Furthermore, the questionnaire used for assessing mindfulness [S25] did not allow differentiating between various facets of mindfulness and, instead, assessed a general mindfulness factor. Thus, it might be possible that using more differentiated measures, such as the CHIME [S26] with an eight-factor structure, could have revealed different association of changes in mindfulness with changes in *SLC6A4* *DNAm*.

Moreover, our results show that the observed changes in SL*C6A4 DNAm* at several CpG sites were inversely associated with changes in *SLC6A4* expression. This pattern was particularly consistent at CpG sites in the promoter region of *SLC6A4*. Although these results did not survive Bonferroni correction, such a constant functional relevance for adjacent CpG sites is unlikely to result from chance. It seems noteworthy that many of these CpG sites (10, 11, 14 – 22) also showed group-specific changes in methylation levels over the course of the study. Given this result, it might be presumed that these CpG sites show an increased susceptibility to changes in methylation levels because these changes serve as mechanisms to adapt serotonergic signaling in response to environmental influences. Overall, these results are in line with basic research showing that methylation of CpG sites close to the TSS blocks the initiation of transcription [S27, S28].However, the overall increase in *SLC6A4* expression in the control group also indicates that alterations in *SLC6A4* expression might also depend on additional biological factors [S28-S30], which we did not assess in this study.

To interpret our results in light of previous studies on changes *in SLC6A4 DNAm* in response to a psychological intervention, a detailed focus on the methods seems necessary: First, in a study by Roberts and colleague [S31], children suffering from anxiety disorders who underwent CBT showed increases in methylation at a *SLC6A4* CpG unit comprising two CpG sites when being classified as responders to CBT. When classified as non-responders, participants showed decreases in methylation at these sites. To the contrary, in our study, participants from the MBI showed a decrease in mean *SLC6A4* *DNAm* following the MBI. These conflicting results might result from differences between both studies: The study populations differed in age and mental health, the interventions were different (MBI vs CBT) and the samples used for extracting genomic DNA were collected using varying methods. In addition, in contrast to the study by Roberts and colleagues [S31], we statistically controlled for possible effects of the *HTTLPR/rs25531* mini haplotype on changes in *SLC6A4 DNAm*. Since an allele-specific *SLC6A4 DNAm* in association with stress has previously been reported [S13, S16 ], we consider this to be a possible reason for the inconsistencies between the study results. Lastly, the method to quantify *SLC6A4 DNAm* (pyrosequencing vs. mass spectrometry) as well as the location of CpG sites differed between both studies. In fact, only CpG site 13 was assessed in both studies. Of note, albeit not statistically significant in the study by Roberts and colleagues [S31], participants free of their primary anxiety diagnosis after the conclusion of the CBT showed a decrease in methylation at this CpG site as compared to non-responders. In another study, Bishop and colleagues [S32] report no differences in changes of *SLC6A4 DNAm* between treatment responders (classified by decreases in PTSD symptom severity), and non-responders in a group of traumatized veterans following mindfulness-based stress reduction. We assume that the discrepancy to our results mainly emerges due to basic characteristics of the two study populations (e.g., traumatized older veterans vs. healthy young participants) and other design choices, such as whether to control for the *HTTLPR/rs25531* mini haplotypeor not. Moreover, out of the 42 CpG sites analyzed in the study by Bishop and colleagues [S32] only 8 CpG sites were also analyzed in our study. It should be added that, while processing the data, the authors used a principal component analyses (PCA) to create a primary component of *SLC6A4 DNAm* comprising 21 CpG sites which was used for all statistical analyses. Thus, albeit methylation in both studies was largely measured in the same genomic region of *SLC6A4*, the factual methylation indices differ profoundly (i.e. mean *SLC6A4 DNAm* together with CpGsite-specific analyses vs. PCA based indicator of *SLC6A4 DNAm*). Another study, which investigated genome-wide alterations in methylation, did not report *SLC6A4* to be differentially methylated in long-term meditators (more than 10 years of practice) as compared to controls [S33]. It is important to consider that the participants of this study did practice another form of mindfulness, the so-called “Open Monitoring Meditation” [S34] which differs from our intervention and demands a higher level of expertise in mindfulness. Furthermore, given the genome-wide approach, the authors were only able to analyze a fraction of the CpG sites we covered. In summary, the inconsistencies in study results can probably be explained by means of the differences in the designs of the studies, which are outline above in detail. Nonetheless, we emphasize that future studies need to avoid these inconsistencies by analyzing the whole CpG island in response to psychological interventions, preferably while parallelizing study designs, such as DNA sampling methods or data processing of methylation values. Correspondingly, it has previously been demanded to address such methodological challenges in future research on *SLC6A4 DNAm* in association with psychological variables [S2].

The present study has several strengths, such as the long-term intervention over the course of three months; CpG site-specific analyses; the possibility to analyze the functional relevance of changes in *SLC6A4 DNAm*; and the in-depth subgroup analyses within the MBI group. However, there are some limitations to be considered when interpreting the results. To address the issue of self-selection, we compared characteristics of both study groups and found them to be equivalent at baseline with regard to almost all covariates and, most importantly, with regard to the main outcome variables. This, thus, prevents biased estimates in our statistical analysis which could occur as a result of unbalanced groups [S35, S36]. To further increase the precision of the intervention-effect estimates, we chose to control for covariates in statistical analysis [S37]. To test the robustness of the intervention effects, we conducted sensitivity analyses which confirmed that neither psychiatric diagnosis nor baseline *SLC6A4 DNAm* did have an impact on group-specific changes in *SLC6A4 DNAm* over the course of the study. In conclusion, our findings were shown to be very robust and not confounded by a wide range of important covariates or measured a priori group differences.

Another limitation is that, given the time-sensitive standard stressor (important exam) that all study participants had to face, a waiting-control group design deemed us not feasible in this study. Also, the sample size is critically small for a conclusive interpretation of the effects of polymorphic variations of *SLC6A4*. This might begin to explain the missing effects of the *5-HTTLPR/rs25531* mini haplotype on changes in *SLC6A4 DNAm* and on changes in *SLC6A4* expression. Furthermore, we acknowledge that a total of 28 CpG sites, which could have been analyzed using the assay for methylation analyses in our study, had to be left out due to limited financial resources. Thus, we have no information on methylation changes at these CpG sites and we cannot rule out that these changes could have altered the results on group-specific changes in mean *SLC6A4 DNAm*. Other methodological issues concerning the usage of peripheral tissues, such as peripheral blood, as a surrogate for tissue within the CNS are seen as controversial. In case of *SLC6A4*, methylation levels measured in peripheral tissue can be translated to methylation levels measured in neural tissue [S28, S38, S39]. Moreover, although significant, the total changes in *SLC6A4 DNAm* observed from pre- to post-intervention remain small. However, such small changes are in line with other studies investigating changes in methylation levels as a result of psychological interventions (e.g., Perroud and colleagues [S40]). Taken together, we conclude that our findings remain valid when considering the limitations as described above. However, given the novelty of these findings, we emphasize the need for replication studies.

**Supplementary References**

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**Appendices**

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| **Table S1**. Primers used for genotyping analyses | |
| Primer name | Primer sequence 5'-3' |
| rs25531\_F | TCCTCCGCTTTGGCGCCTCTTCC |
| rs25531\_R | TGGGGGTTGCAGGGGAGATCCTG |

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| **Table S2.** Primer sequences for mRNA expression analyses | | | |
| Primer name | Primer sequence 5'-3' |  |  |
| 5HTT-F | GTGATTGGCTATGCTGTGGA |  |  |
| 5HTT-R | ATGGTGTAGGGGAGGAGGAA |  |  |
| ACTB-F | GTGGGCATGGGTCAGAAG |  |  |
| ACTB-R | GATGGGGTACTTCAGGGTGAG |  |  |
| GAPDH-F | TGAGTACGTCGTGGAGTCCA |  |  |
| GAPDH-R | ATGTTCGTCATGGGTGTGAA |  |  |

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| **Table S3.** Amplicon primers and sequencing primers used for bisulfite pyrosequencing within the 799-bp promoter-associated CpG island of *SLC6A4* | | | | |
|  | Amplicon primer name | Amplicon primer  sequence 5'-3' | Sequencing primer name | Sequencing primer sequence 5'-3' |
| Amplicon 1 (CpG sites  8-13) | 5HTT\_P1-F | ggg ttt tta agt tga gtt tat at | 5HTT\_P1-S2 (CpG 8-13) | gag tag att ttt gtg tg |
| 5HTT\_P1-R | Biotin-cta act ttc cta ctc ttt aac tt |  |  |
| Amplicon 2 (CpG sites 14-22) | 5HTT\_P2-F | aag agt agg aaa gtt agg a | 5HTT\_P2-S1 (CpG 14-22) | gta gga aag tta gga ttt |
| 5HTT\_P2-R | Biotin-ccc tca cat aat cta atc t |  |  |
| Amplicon 3 (CpG sites 43-83) | 5HTT\_P3-F | ggg gaa gta tta agt tta t | 5HTT\_P3-S1 (CpG 43-57) | att tag aga tta gat tat gtg |
| 5HTT\_P3-R | Biotin-ccc cta caa caa taa aca | 5HTT\_P3-S2 (CpG 58-64) | agg tta gtt agt ttg ttt ag |
|  |  | 5HTT\_P3-S3 (CpG 65-71) | att taa gtt ttt ttt tag at |
|  |  | 5HTT\_P3-S4 (CpG 72-77) | agg aga gga ggt gta t |
|  |  | 5HTT\_P3-S5 (CpG 78-83) | tta gta aga gtt aga gtt gaa |
| All described sequences refer to bisulfite treated DNA. | | | | |

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| **Table S4.** CpG sites of *SLC6A4* covered by frequently used microarrays in relation to CpG sites of *SLC6A4* covered in the pyrosquencing analyses of the present study | | |
| IlmnID-450k | IlmnID-EPIC | # of CpG site in Stoffel et al. |
| cg27569822 |  | 8 |
| cg27569822 | cg10901968 | 9 |
| cg26741280 | cg26741280 | 12 |
| cg25725890 | cg25725890 | 13 |
| cg05016953 | cg05016953 | - |
|  | cg06373684 | 43 |
|  | cg26438554 | 46 |
| cg14692377 | cg14692377 | 55 |
| cg03363743 | cg03363743 | 76 |

**O:\Psychobiologie_Stress\MISC\Manuskript_Bio._Psychiatry\Finale Versionen zur Einreichung\Grafiken\Figure_S1.tifFig. S1.** The base sequences of the analyzed region within the 799-bp promoter-associated CpG island of *SLC6A4* ocomprising 534 base pairs. The analyzed CpG sites are numbered accordingly and highlighted in red. CpG sites within the sequence shown in grey were not analyzed. Base positions in compliance with the NCBI genome browser (GenBank accession number: NG\_011747.2) are shown on the left.

**O:\Psychobiologie_Stress\finale Versionen zum Einreichen\Figure_S2_greyscale.tifFig. S2.** Boxplots showing mean methylation for 56 analyzed CpG sites of *N* = 74 participants (mean ± SD).

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| **Table S5.** Baseline sample characteristics | | | | | |
|  | MBI group | control group | Group comparisons1 | | |
| Stat. | df | *p*-value |
| *Sex* |  |  |  |  |  |
| Female | *n* = 18 (64.3%) | *n* = 30 (65.2%) | *χ²* = 0 | 1 | 1.000 |
| *Age* |  |  |  |  |  |
| mean (SD) | 20.79 (1.57) | 21.41 (3.69) | *t* = 1.013 | 66.081 | 0.3147 |
| *Body mass index* |  |  |  |  |  |
| mean (SD) | 20.85 (2.05) | 22.62 (3.05) | *t* = 2.9862 | 71.181 | 0.0039 |
| *Smoking* |  |  |  |  |  |
| Smokers | *n* = 7 (25%) | *n* = 7 (15.2%) | *χ²* = 0.5418 | 1 | 0.4617 |
| mean (SD) of cigarettes smoked per day | 2.9 (0.96) | 5.14 (6.05) | *t =* 0.9645 | 6.4205 | 0.3697 |
| *Hormonal contraception* |  |  |  |  |  |
| Yes | *n* = 9 (32.1%) | *n* = 19 (41.3%) | *χ²* = 0.2927 | 1 | 0.5885 |
| *Recent life event* |  |  |  |  |  |
| Yes | *n* = 13 (46.4%) | *n* = 28 (60.9%) | *χ²* = 0.9427 | 1 | 0.3316 |
| *Mindfulness (FMI score)* |  |  |  |  |  |
| mean (SD) | 36.36 (4.24) | 37.91 (4.86) | *t* = 1.4435 | 63.284 | 0.1538 |
| *Childhood adversities (CTQ score)* |  |  |  |  |  |
| mean (SD) | 32.54 (7.32) | 33.2 (9.24) | *t* = 0.3399 | 66.966 | 0.735 |
| *Chronic stress (TICS score)* |  |  |  |  |  |
| mean (SD) | 18.32 (5.96) | 20.17 (8.29) | *t* = 1.1141 | 69.881 | 0.2691 |
| *Mean SLC6A4 DNAm (percentage)* |  |  |  |  |  |
| mean (SD) | 9.79 (0.8) | 9.85 (0.94) | *t* = 0.2968 | 64.467 | 0.7675 |
| *HTTLPR/rs255312* |  |  |  |  |  |
| LL | *n =* 10 (35.7%) | *n* = 14 (30.4%) | - | - | - |
| LS | *n* = 8 (28.5%) | *n* = 24 (52.2%) | - | - | - |
| SS | *n* = 8 (28.5%) | *n* = 7 (15.2%) | - | - | - |
| Unknown | *n* = 2 (7.1%) | *n* = 1 (2.2%) | - | - | - |
| *Psychiatric diagnoses* |  |  |  |  |  |
| Depressive disorder | *-* | *n*= 2 (4.3%) | - | - | - |
| Anxiety disorder | *n*= 1 (3.6%) | - | - | - | - |
| ADHD | - | *n* = 1 (2.2%) | - | - | - |
| Sleep disorder | - | *n* = 1 (2.2%) | - | - | - |
| *Ethnicity* |  |  |  |  |  |
| White European ancestry for at  least two generations | *n*= 25 (89.3%) | *n*= 35 (76.1%) | - | - | - |
| Other ethnicity | *n*= 2 (7.1%) | *n*= 8 (17.4%) | - | - | - |
| Unknown | *n =* 1 (3.6%) | *n =* 3 (6.5%) | - | - | - |
| FMI, Freiburg Mindfulness Inventory; CTQ, Childhood Trauma Questionnaire; TICS, Trier Inventory for the Assessment of Chronic Stress; *SLC6A4 DNAm*, CpG methylation in a *SLC6A4* promoter-associated 799-bp CpG island; ADHD, attention deficit hyperactivity disorder.  1Where applicable, *p*-values were obtained from significance tests conducted to compare baseline characteristics between groups. *χ*² tests were used in case of categorical variables while Welch’s t-tests were used to compare continuous variables.  2Biallelic classification of the *5-HTTLPR/rs25531* mini haplotype. | | | | | |

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| **Table S6.** Results of the retrospective questionnaire assessed in the MBI group | |
|  | Descriptive statistics |
| *Excercising of mindfulness practices in*  *everday life throughout the intervention* |  |
| Yes | *n* = 12 (42.9%) |
| No | *n* = 15 (53.6%) |
| Unknown | *n* = 1 (3.6%) |
| *Increased ability to cope with stress* |  |
| Yes | *n* = 20 (71.4%) |
| No | *n* = 5 (17.9%) |
| Unknown | *n* = 3 (10.7%) |
| *Reduction of stress*1 |  |
| mean (SD) | 3.58 (2.25) |
| *Feasibility of mindfulness practices*2 |  |
| mean (SD) | 3.81 (2.42) |
| 1Visual analogue scales were used to rate the extent to which MBI participants felt a reduction of stress (0 = not at all, 10 = very high). Data are missing for three participants. 2Visual analogue scales were used to rate the feasibility of the mindfulness practices (0 = not at all, 10 = very good). Data are missing for three participants. | |

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| **Table S7.** Comparison of methylation changes at the 56 analyzed CpG sites of *SLC6A4* between the MBI group and the control group | | | | |
|  | Differences in methylation change from pre- to post-intervention | | CpG site-specific change scores (percentage point changes1) | |
| Group-by-time interaction; *b* (SE) | *p*-value | MBI group; mean (SD) | control group; mean (SD) |
| CpG8 | -2.8311 (0.777) | **0.0006\*\*** | -2.9367 (4.9968) | -0.1918 (2.6549) |
| CpG9 | -0.9194 (0.2763) | 0.0015\* | -0.7111 (1.5185) | 0.2378 (0.7942) |
| CpG10 | -1.6197 (0.4444) | **0.0006\*\*** | -2.1367 (2.1864) | -0.1173 (1.4494) |
| CpG11 | -1.1618 (0.2523) | **<0.0001\*\*\*** | -0.8593 (1.2787) | 0.4478 (0.8245) |
| CpG12 | -0.8087 (0.2826) | 0.0058\* | -0.5896 (2.1482) | 0.0396 (0.9553) |
| CpG13 | -2.8144 (0.385) | **<0.0001\*\*\*** | -0.78 (4.4505) | -0.0243 (1.8233) |
| CpG14 | -5.9309 (0.8199) | **<0.0001\*\*\*** | -3.8248 (7.4363) | 2.6342 (6.731) |
| CpG15 | -4.1081 (0.7014) | **<0.0001\*\*\*** | -2.3967 (3.8822) | 1.6038 (2.769) |
| CpG16 | -3.4414 (0.6528) | **<0.0001\*\*\*** | -1.8689 (3.594) | 1.3418 (2.2984) |
| CpG17 | -4.1644 (0.8187) | **<0.0001\*\*\*** | -2.1304 (3.7416) | 2.0132 (2.7752) |
| CpG18 | -2.7866 (0.528) | **<0.0001\*\*\*** | -1.1777 (2.8631) | 1.6293 (2.0423) |
| CpG19 | -3.4068 (0.8244) | **<0.0001\*\*\*** | -1.5107 (4.4075) | 1.9507 (2.9283) |
| CpG20 | -2.0373 (0.541) | **0.0004\*\*** | -0.4954 (2.8315) | 1.3798 (1.7914) |
| CpG21 | -3.6606 (0.7417) | **<0.0001\*\*\*** | -1.6815 (3.9903) | 2.0231 (2.3464) |
| CpG22 | -4.7622 (0.7054) | **<0.0001\*\*\*** | -2.6435 (3.4749) | 1.652 (2.5704) |
| CpG43 | -1.7449 (0.5155) | 0.0013\* | -1.2067 (2.3652) | 0.6262 (2.3385) |
| CpG44 | 0.7601 (0.3306) | 0.0251\* | -0.1022 (1.4819) | -0.6664 (1.2743) |
| CpG45 | 0.5749 (0.3621) | 0.1177 | -0.2685 (1.5638) | -0.6547 (1.4038) |
| CpG46 | 1.482 (0.4481) | 0.0016\* | -0.0907 (1.7403) | -1.6147 (1.6836) |
| CpG47 | 1.2369 (0.3577) | 0.001\* | -0.093 (1.7334) | -0.9929 (1.5677) |
| CpG48 | 2.0653 (0.3688) | **<0.0001\*\*\*** | -0.0174 (1.6481) | -1.902 (1.5576) |
| CpG49 | 1.2279 (0.392) | 0.0027\* | 0.0259 (1.3996) | -1.1776 (1.5023) |
| CpG50 | 1.2614 (0.5899) | 0.0366\* | -0.5907 (2.074) | -1.9762 (2.4605) |
| CpG51 | 0.1428 (0.4372) | 0.7451 | -0.6893 (2.0422) | -1.044 (1.8124) |
| CpG52 | 0.8904 (0.5192) | 0.0916 | -0.3726 (2.4726) | -1.3142 (1.6584) |
| CpG53 | 2.3186 (0.5981) | **0.0003\*\*** | -0.3667 (2.8452) | -2.3062 (2.2944) |
| CpG54 | 1.8719 (0.5802) | 0.002\* | 0.4396 (2.6791) | -1.4878 (1.9268) |
| CpG55 | 1.6586 (0.5574) | 0.0043\* | 0.5296 (2.0494) | -1.1969 (2.0988) |
| CpG56 | 1.5146 (0.5921) | 0.0132\* | 0.4388 (2.8709) | -1.0278 (1.9889) |
| CpG57 | 1.666 (0.8397) | 0.0521 | 0.2468 (5.855) | -2.2484 (2.486) |
| CpG58 | 1.7409 (0.6311) | 0.008\* | 1.0382 (2.2662) | -0.7443 (2.3851) |
| CpG59 | 1.3055 (0.5943) | 0.032\* | 0.0885 (2.2734) | -0.688 (2.2594) |
| CpG60 | 1.8774 (0.5401) | 0.001\* | 1.6537 (2.1471) | 0.1909 (1.9444) |
| CpG61 | 0.4049 (0.6236) | 0.5187 | -0.0833 (2.534) | -0.324 (2.2835) |
| CpG62 | 0.2446 (0.7908) | 0.7582 | -0.4619 (2.805) | -0.6663 (2.974) |
| CpG63 | -0.1898 (0.4182) | 0.6517 | -0.4741 (1.6922) | -0.4378 (1.6025) |
| CpG64 | 0.1687 (0.2309) | 0.468 | -0.1778 (0.6948) | -0.3425 (1.1082) |
| CpG65 | -3.6058 (0.8803) | **0.0002\*\*** | -3.4972 (2.9601) | 0.4747 (3.6341) |
| CpG66 | -1.7121 (0.5768) | 0.0044\* | -1.4267 (2.2087) | 0.5616 (3.3761) |
| CpG67 | -0.6346 (0.6704) | 0.3477 | -1.4874 (3.4647) | 0.2616 (5.4325) |
| CpG68 | -1.2667 (0.6252) | 0.0475\* | -0.8159 (2.3211) | 0.6288 (2.6135) |
| CpG69 | -0.6334 (0.854) | 0.4612 | -1.3007 (3.251) | -0.4651 (4.399) |
| CpG70 | -1.3165 (0.6111) | 0.0354\* | -1.3619 (2.364) | 0.2559 (2.9219) |
| CpG71 | 0.0931 (0.6753) | 0.8909 | -0.5488 (3.0907) | -0.1673 (2.9284) |
| CpG72 | -0.6736 (0.5192) | 0.1995 | 0.707 (1.7571) | 1.1462 (2.4816) |
| CpG73 | -1.793 (0.566) | 0.0024\* | -0.6981 (2.5853) | 0.5744 (2.3603) |
| CpG74 | 0.868 (0.5827) | 0.1417 | 1.2919 (2.1248) | 0.4687 (2.3335) |
| CpG75 | -0.2051 (0.485) | 0.6739 | -0.5441 (2.2241) | -0.5171 (1.7768) |
| CpG76 | 0.5372 (0.6177) | 0.388 | 0.67 (2.9646) | 0.1878 (1.8611) |
| CpG77 | 1.3572 (0.5527) | 0.017\* | 0.4156 (1.8247) | -0.5644 (2.2338) |
| CpG78 | 0.9276 (0.5821) | 0.1163 | 1.6159 (2.9449) | -0.856 (2.5502) |
| CpG79 | 1.1477 (0.6812) | 0.0975 | 1.6493 (3.0482) | 0.2744 (8.1062) |
| CpG80 | 0.5057 (0.7524) | 0.5042 | 2.0833 (3.3383) | 0.5216 (8.2811) |
| CpG81 | 0.7072 (0.6357) | 0.2706 | 1.1426 (2.2464) | -0.0077 (7.5824) |
| CpG82 | 0.1484 (0.8638) | 0.8642 | -0.6204 (2.7931) | -0.5784 (10.4225) |
| CpG83 | -0.1172 (0.8647) | 0.8926 | -0.7733 (3.6781) | 0.6463 (9.103) |
| Group-by-time interactions and their *p*-values were obtained from multilevel models analyzing group-specific changes in methylation from pre- to post-intervention. \*significant at *p*< 0.05; \*\*significant at *p* < 0.001; \*\*\*significant at *p* < 0.0001. Bold *p*-values are significant after Bonferroni correction for multiple testing (*p* < 0.0009).  1Percentage point changes were calculated as: methylation post-intervention – methylation pre-intervention. | | | | |

**O:\Psychobiologie_Stress\finale Versionen zum Einreichen\Figure_S3_greyscale.tifFig. S3.** Group-specific changes in CpG site-specific methylation in a *SLC6A4* promoter-associated 799-bp CpG island from pre- to post-intervention. Changes in methylation are shown for participants in the MBI group (black bars, mean ± SE) and in the control group (white bars, mean ± SE). Group-specific changes in methylation for each CpG site from pre- to post-intervention were analyzed using multilevel models with group-by-time interactions; \*significant at *p* < 0.05. Percentage point changes were calculated as: methylation post-intervention – methylation pre-intervention.

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| **Table S8.** Associations of sex with *SLC6A4* CpG site-specific methylation | | |
|  | b (SE) | *p*-value |
| CpG 62 | 1.2568 (0.5217) | 0.0188\* |
| CpG 69 | 1.5173 (0.7233) | 0.0398\* |
| CpG 71 | 1.8191 (0.716) | 0.0135\* |
| CpG 72 | 1.4948 (0.5247) | 0.0059\* |
| CpG 73 | 1.8399 (0.5228) | 0.0008\*\* |
| CpG 74 | 1.3333 (0.4546) | 0.0046\* |
| CpG 75 | 1.3818 (0.4966) | 0.0071\* |
| CpG 76 | 2.726 (0.616) | < 0.0001\*\*\* |
| CpG 77 | 3.3275 (0.6771) | < 0.0001\*\*\* |
| CpG 78 | 2.5598 (0.6197) | 0.0001\*\* |
| CpG 79 | 2.3881 (0.7031) | 0.0012\* |
| CpG 81 | 2.5981 (0.775) | 0.0014\* |
| CpG 82 | 4.667 (1.1589) | 0.0002\*\* |
| CpG 83 | 7.6854 (1.1694) | < 0.0001\*\*\* |
| \*significant at *p* < 0.05; \*\*significant at *p* < 0.001; \*\*\*significant at *p* < 0.0001. CpG site-specific methylation was higher for women than for men for all CpG sites depicted in this table. Associations were obtained in multilevel models. In each model, sex was entered as predictor to control for the variance in CpG site-specific methylation attributable to sex. | | |

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| **Table S9.** Pearson´s product-moment correlations for the relationship of fold changes in *SLC6A4* expression and changes in methylation significant at the level of *p* ≤ 0.05 (uncorrected for multiple comparisons) | | |
|  | Fold changes in *SLC6A4* expression | |
| CpG site-specific change scores (percentage point changes) | Correlation coefficient | *p-*value |
| CpG9 | -0.2682 | 0.0227 |
| CpG10 | -0.3053 | 0.0091 |
| CpG11 | -0.3211 | 0.006 |
| CpG14 | -0.3162 | 0.0068 |
| CpG15 | -0.3682 | 0.0015 |
| CpG16 | -0.3646 | 0.0016 |
| CpG17 | -0.3326 | 0.0046 |
| CpG18 | -0.2924 | 0.0155 |
| CpG19 | -0.3502 | 0.003 |
| CpG20 | -0.2505 | 0.0409 |
| CpG21 | -0.3423 | 0.004 |
| CpG22 | -0.28 | 0.025 |
| CpG43 | -0.2714 | 0.0211 |
| CpG57 | 0.2599 | 0.0298 |
| CpG62 | -0.2394 | 0.0476 |
| Lower methylation change scores indicate a higher decrease in methylation from pre- to post-intervention at the respective CpG site. Higher fold changes in *SLC6A4* expression are indicative of an increase in *SLC6A4* expression. | | |