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Transcendental Meditation practitioners show reduced expression of the Conserved Transcriptional Response to Adversity

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ABSTRACT

Background and objectives: A recent exploratory study of transcriptional effects of long-term practice of Transcendental Meditation (TM) technologies found evidence for altered expression of genes associated with health and disease. In the present secondary analysis of those data, we test the more specific hypothesis that this sample of long-term practitioners shows a significant reduction in markers of the "Conserved Transcriptional Response to Adversity" (CTRA), an RNA profile characterized by up-regulated inflammation and down-regulated Type I interferon (IFN) activity.

Materials and methods: Data come from a previously published study providing genome-wide transcriptional profiles of peripheral blood mononuclear cells (PBMC) from healthy, 38-year practitioners of TM technologies and matched controls ($n = 12$, mean age 65). The current analysis specifically tests for differential expression of a previously established CTRA indicator gene score, with cross-validation by promoter-based bioinformatic analysis of CTRA-typical differences in transcription factor activity and monocyte subset cellular origins.

Results: Compared to controls, the TM group showed lower expression of a pre-specified set of CTRA indicator genes. These effects were accompanied by genome-wide indications of down-regulated pro-inflammatory transcription factor activity (NF-κB, AP-1), up-regulated activity of Interferon Response Factors (IRF) and reduced transcriptional activity of classical monocytes. *Conclusions*: A sample of long-term practitioners of TM showed reduced CTRA gene expression in PBMC compared to matched controls, supporting the likely value of further research to evaluate causality and specificity of this potential mechanism of health benefits in meditators.

1. Introduction

The history of research on the Transcendental Meditation (TM) technique has followed 2 main thrusts. In the first, studies have addressed the question of what happens within the practice to distinguish it from eyes closed rest and from other mental practices [see e.g., (Wallace, 1970; Jevning et al., 1978; Travis and Pearson, 2000; Travis and Shear, 2010; Mahone et al., 2018)]. In the second, a larger number of studies have reported associations between the twice-daily practice of TM and benefits for individual and collective health [see e.g., (Schneider et al., 2012; Paul-Labrador et al., 2006; Orme-Johnson, 1987; Dillbeck and Cavanaugh, 2023)]. The current secondary analysis of data from a previous report (Wenuganen et al., 2021) concerns a less-pursued but important topic in TM research, namely, the search for biological mechanisms capable of supporting the reported health and wellbeing benefits of the practice.

In considering possible mechanisms, an ability to reduce damaging effects of psychosocial stress and other adversity is a prime candidate. The first study of effects of TM suggesting the technique might reduce effects of stress found an acute increase in metabolic efficiency, stabilized galvanic skin response, and EEG patterns indicating a novel, restfully alert state different from eyes closed rest (Wallace, 1970). Regular practice also has been linked to reductions in excessive hypothalamic-pituitary-adrenal (HPA) axis activity and other long-lasting effects, such as PTSD, that can result from chronic or extreme acute stress (MacLean et al., 1997; Nidich et al., 2018). These

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physiologic alterations related to stress adaptation may contribute to health benefits previously connected with the practice, as evidenced, for example, by reduced health insurance utilization (Orme-Johnson, 1987; Herron, 2011; Herron and Hillis, 2000) and by reductions in cardiovascular disease (Schneider et al., 2012, 2019; Paul-Labrador et al., 2006; Castillo-Richmond et al., 2000) and its risk factors [see for review (Walton et al., 2002)].

Psychosocial adversity also impacts the immune system and does so in ways that negatively affect health. Little is known about the impact of TM on immune system function, but a recent exploratory study conducted descriptive analyses of genome-wide transcriptome profiles of peripheral blood mononuclear cells (PBMC) collected from long-term TM practitioners and matched controls (Wenuganen et al., 2021). In that study, Gene Ontology and Canonical Pathway analyses linked TM practice to altered activity of multiple gene sets involved in inflammation, innate immunity, and hematopoiesis. Due to the exploratory nature of the data analyses, these effects appeared complex, being characterized by both up- and down-regulation of inflammation- and immune-relevant pathways in long-term TM practitioners. However, some of the transcriptomic effects in that study suggested a possible effect on the Conserved Transcriptional Response to Adversity (CTRA), an RNA profile previously documented to arise from sympathetic nervous system-induced beta-adrenergic signaling and its effects in promoting hematopoiesis of myeloid lineage immune cells (monocytes, neutrophils, dendritic cells) and biasing the aggregate circulating leukocyte transcriptome toward greater inflammatory activity and reduced innate antiviral activity (i.e., Type I interferon; IFN) (Cole, 2013, 2019). The descriptive "discovery-based" approach of the previous paper leaves it unclear whether these transcriptomic differences associated with long-term TM practice represented a significant quantitative reduction in markers of the CTRA profile.

To address this question, we conducted a hypothesis-driven reanalysis of the previously published transcriptomic data. This analysis directly tests whether the transcriptomic correlates of long-term TM practice manifest lower levels of 3 well-established indicators of CTRA gene regulation (Cole, 2019; Cole et al., 2020): 1) expression of an *a priori*-specified CTRA contrast score defined by elevated expression of cardinal pro-inflammatory gene transcripts (e.g., *IL1B, IL6, IL8, TNF, PTGS2*) and reduced expression of cardinal Type I IFN response gene transcripts (e.g., *IFI-, MX-,* and *OAS*-family genes); 2) CTRA-characteristic patterns of transcription factor activity as inferred from bioinformatic analysis of unbiased (genome-wide) differences in gene expression (i.e., increased activity of pro-inflammatory NF-kB and AP-1 transcription factors and reduced activity of IRF family factors); and 3) CTRA-characteristic patterns of cellular activity, as inferred from transcript origin analysis of unbiased (genome-wide) differentially expressed genes (i.e., increased activity of classical monocytes).

2. Materials and Methods

This study represents a secondary analysis of publicly posted PBMC transcriptome profiling data from 12 individuals, including 6 healthy long-term (38-year) TM practitioners and 6 matched healthy control participants, as previously described (Wenuganen et al., 2021). Details of participant selection and demographic matching are provided under Materials and Methods of that open-source publication, and involve matching on age and sex, with all participants homogenous on race (self-identified White), general lifestyle parameters (non-vegetarian, non-smoker, non-drinker, moderate exercise), absence of diagnosed diseases (specifically, diabetes, nerve damage, heart attack, coronary heart disease, stroke, kidney failure, cancer, any other life-threatening illness, a major psychiatric disorder, or substance abuse), and subjective socioeconomic status. The meditation program [see (Wenuganen et al., 2021)] consisted of 2 parts. The standard TM technique (the first part) is an effortless, eyes-closed, inward mental procedure in which a select sound called a "mantra" is used in a manner that promotes "automatic transcending," where the mind automatically moves or "transcends" to finer levels of the mantra and finally beyond the thinking process itself (Travis and Pearson, 2000; Travis and Shear, 2010). This part was practiced twice daily for (mean ± SD) 458 ± 49 months. The second part, added later, was the related, eyes-closed, TM-Sidhi mental procedure, also practiced twice daily for the last 406 ± 50 months. Neither of these components involves conscious control of breathing or movement. Ancillary practices such as Yoga asanas (positions) and alternate-nostril breathing are recommended but are not part of the prescribed program.

PBMC were isolated by density gradient centrifugation (BD Vacutainer® CPT™ Mononuclear Cell Preparation Tube), with RNA extracted (RNAzol B Kit; Ambion®), tested for suitable quantity and quality, and converted to labeled cDNA (Illumina TotalPrep™ RNA Labeling Kit), which was hybridized to Illumina HumanHT-12v4 microarrays, and imaged using the Illumina iScan instrument, with all microarray procedures performed by the University of Chicago Genomics Core Laboratory following the manufacturer's standard protocol.

For the present analyses, background-corrected quantile-normalized probe-level gene expression data were downloaded from the public EBI ArrayExpress database (E-MTAB-10252), log2-transformed for linear statistical model analyses (with negative background-corrected values in the original data set to 0), and screened to exclude transcripts that were not consistently detectable above background or showed little differential expression between conditions (i.e., difference < 1 SD). Standard linear statistical model analyses then quantified the difference in average expression of each transcript in PBMC from TM vs control samples while controlling for participant sex (which was slightly imbalanced, with males comprising 5 of 6 TM practitioners and 4 of 6 controls). Note that estimates of differential gene expression derived from these analyses will differ from those of the previous discovery analysis report due to this analysis controlling for participant sex, excluding probes with minimal variation in expression, and using point estimates of differential gene expression as input into higher-order bioinformatics analyses, an approach yielding more reliable results than *p*-value-curated gene lists (Cole et al., 2003).

Our primary analysis followed previous research (Cole et al., 2020) in assessing the CTRA profile using an *a priori*-defined contrast among gene expression values for 19 pro-inflammatory genes (*IL1A, IL1B, IL6, IL8, TNF,PTGS1, PTGS2, FOS, FOSB, FOSL1, FOSL2, JUN, JUNB, JUND, NFKB1, NFKB2, REL, RELA, RELB*), 28 genes involved in Type I interferon response (*GBP1, IFI16, IFI27, IFI27L1-2, IFI30, IFI35, IFI44, IFI44L, IFI6, IFIH1, IFIT1-3, IFIT5,IFIT1L, IFITM1-3, IFITM4P, IFITM5, IFNB1, IRF2, IRF7-8, MX1-2, OAS1-3, OASL*), and 3 genes involved in antibody synthesis (*IGJ, IGLL1, IGLL3*), with signs reversed for the antiviral and antibody-related gene sets to reflect their inverse relationship to the CTRA profile (Cole, 2019; Cole et al., 2020). In the event of significant group differences in the overall CTRA composite score, we conducted follow-up tests of the pro-inflammatory and antiviral gene sets separately to determine which subcomponents might contribute to the composite score difference.

To test the results of primary analyses using alternative methods, we assessed genome-wide differences in transcript abundance for TM practitioners *vs*. controls and conducted higher-order promoter-based bioinformatic analyses of those empirical differences to determine whether they showed differential activity of CTRA-related transcription factors (i.e., increased NF-κB and AP-1 activity and reduced IRF activity) or cellular activation (i.e., increased activity of classical monocytes *vs*. non-classical monocytes). All the CTRA measures used here are continuous scales, with solely relative interpretation across TM and control groups, thus disallowing CTRA "scores" to be calculated for individuals. In these analyses, all gene transcripts showing $>$ 2-fold difference in average expression level in PBMC from TM practitioners *vs*. controls served as input into TELiS promoter-based bioinformatics analyses quantifying relative prevalence of NF-κB, AP-1, and IRF transcription factor-binding motifs (TFBMs) in the core promoter sequences

of up- *vs*. down-regulated genes (Cole et al., 2005, 2020). Analyses used the JASPAR NFKB2 and FOS::JUN, and TRANSFAC V$IRF1_01 position-specific weight matrices to detect TFBMs in core promoter sequences characterized by 9 combinations of 3 different core promoter lengths (-300, -600, and -1000 to +200 nucleotides relative to the RefSeq transcription start site) and 3 different TFBM detection stringencies (TRANSFAC mat_sim values of 0.80, 0.90, and 0.95). Log2 TFBM ratios were computed for each parametric combination and averaged to provide a pooled effect estimate, with that effect tested for statistically significant deviation from the null hypothesis value of 0 using bootstrap resampling of linear model residual vectors (which controls for residual correlation across genes). Parallel Transcript Origin Analysis (Cole et al., 2011) annotated the same set of differentially expressed genes using pre-specified cell type diagnosticity scores derived from previous transcriptome profiling analysis of CD16(−) classical monocytes *vs.* CD16 (+) non-classical monocytes (Wong et al., 2011). To determine whether any differences in cellular origins of transcriptome differences might stem from differential abundance of classical *vs*. non-classical monocytes in TM *vs.* controls, we conducted an additional Transcriptome Representation Analysis (Powell et al., 2013) testing whether the genes that showed > 6 SD differential expression in classical *vs.* non-classical monocytes in the reference study (Wong et al., 2011) also showed differential average expression in TM practitioners *vs.* controls. Again, statistical testing was based on bootstrapped standard error estimates controlling for correlation among genes.

3. Results

3.1. CTRA contrast score

Compared to matched controls, PBMC from long-term TM practitioners showed significantly lower expression of a pre-specified composite of CTRA indicator gene transcripts (difference in average log2 mRNA abundance = -0.34 ± 0.14, $p = .003$; Fig. 1A, left bar). In follow-up analyses of separate inflammatory and antiviral subcomponents of the CTRA composite (Fig. 1A, right 2 bars), results showed a significant up-regulation of the Type I IFN antiviral sub-component of the CTRA composite score (+0.79 ± 0.29, $p = .009$) but no significant difference in the pro-inflammatory component (+0.14 ± 0.17, $p = .419$).

3.2. Transcription factor activity

To provide an alternative assessment of CTRA biology via empirical differences in genome-wide transcriptional profiles, we identified all genes showing >2-fold difference in average expression in TM practitioners *vs*. controls (1,764 total; 866 relatively up-regulated in TM practitioners and 897 relatively down-regulated) and entered those transcripts into TELiS promoter-based bioinformatics analysis. Results (Fig. 1B) indicated significant down-regulation of the pro-inflammatory NF-κB (mean log2 TFBM ratio = -1.02 ± 0.18, $p < .001$) and AP-1 (-0.45 ± 0.18, $p = .016$) transcription control pathways in TM practitioners *vs.* controls, as well as elevated IRF activity (0.87 ± 0.30, $p = .003$).

3.3. Cellular origins

In a second alternative assessment of CTRA biology via bioinformatic analyses of cellular mechanisms, we entered the same sets of up- and down-regulated genes into Transcript Origin Analyses assessing relative activity of classical (CD16(−)) *vs.* non-classical (CD16(+)) monocytes (Fig. 2A). The 897 TM-down-regulated transcripts were identified as deriving predominantly from classical monocytes (mean cell type diagnosticity score = .061 ± .031, $p = .024$), whereas TM-up-regulated genes derived predominately from non-classical monocytes (.080 ± .047, $p = .047$). To determine whether these differences might reflect differential prevalence of these monocyte subsets, Transcriptome Representation Analyses tested for TM-associated differences in expression of 13 genes showing > 6 SD up-regulation in classical *vs.* non-classical

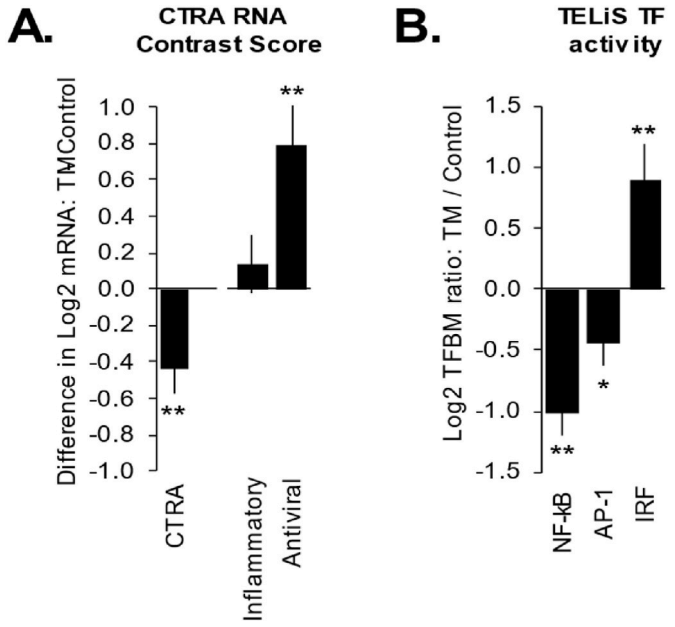

Fig. 1. CTRA gene regulation in long-term TM practitioners *vs*. controls. (A) Difference between TM practitioners and controls in average expression of a pre-specified multi-transcript CTRA composite score (inflammatory – antiviral; left bar), and separate inflammatory and antiviral subcomponents (right 2 bars). (B) Difference between TM practitioners and controls in pro-inflammatory transcription factor activity (NF-kB, AP-1) and antiviral transcription factor activity (IRF) as inferred from promoter-based bioinformatics analysis of genome-wide transcriptional profiles. Data represent the mean ± standard error, with statistical significance levels *$p < .05$, **$p < .01$, ***$p < .001$.

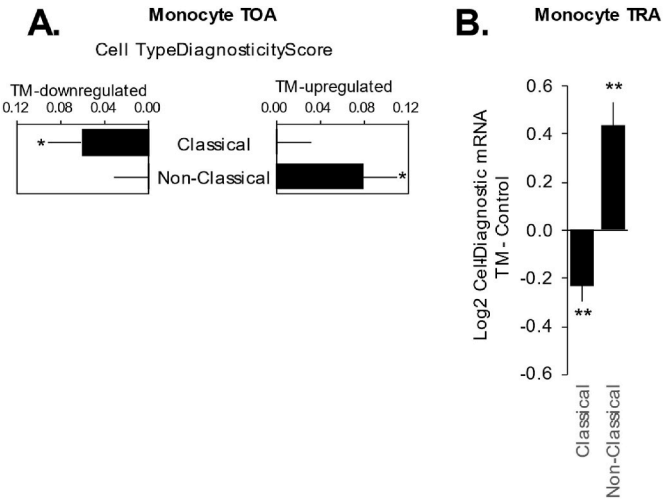

Fig. 2. Cellular sources of differential gene expression in long-term TM practitioners *vs*. controls. (A) Contribution of classical (CD16−) and non-classical (CD16+ monocytes) to TM-related differences in genome-wide transcriptional profiles, as inferred from Transcript Origin Analyses of genes showing empirical up-regulation (right panel) or down-regulation (left panel) in TM practitioners *vs*. controls. (B) Differential abundance of gene transcripts diagnostic of classical monocytes (left bar) and non-classical monocytes (right bar) in Transcriptome Representation Analyses of genome-wide transcriptional profiles of PBMC from TM practitioners *vs*. controls. Data represent the mean ± standard error, with statistical significance levels *$p < .05$, **$p < .01$, ***$p < .001$.

monocytes in a previously published reference study (Wong et al., 2011) and 12 genes showing > 6 SD up-regulation in non-classical *vs*. classical monocytes (Fig. 2B). In these analyses, classical monocyte-characteristic genes were significantly down-regulated in TM *vs.* control (-.229 ± .069 log2 mRNA abundance, $p = .006$) and non-classical monocyte-diagnostic genes were significantly up-regulation in TM *vs.* control (0.441 ± .093, $p < .001$).

4. Discussion

In this analysis of PBMC transcriptome profiles from a sample of long-term TM practitioners and matched healthy controls, hypothesis-testing bioinformatic analyses linked TM practice to multiple indications of reduced CTRA gene regulation, including 1.) reductions in a pre-specified composite score capturing the CTRA pattern of up-regulated inflammatory gene expression and down-regulated Type I IFN antiviral transcript abundance; 2.) down-regulation of pro-inflammatory transcription factor activity (NF-κB, AP-1) and up-regulation of interferon response factor (IRF) activity in genome-wide empirical transcriptomic correlates of TM; and 3.) down-regulation of classical monocyte abundance and activity, and up-regulation of non-classical monocyte abundance and activity.

These transcriptional correlates of TM practice are consistent with a possible role of reduced CTRA gene expression in mediating some of the previously reported health benefits of the TM technique. They are consistent also with findings from randomized controlled trials examining the effects of other meditative practices on CTRA gene expression (Cole, 2019). For example, Black and colleagues prospectively randomized 45 dementia caretakers to either 8 weeks of Yogic meditation or relaxing music and found similar signs of CTRA down-regulation in the meditation group (Black et al., 2013). That Yogic meditation technique used repetition of sounds in a manner somewhat like the TM technology used here, which is said to promote a "transcendental consciousness" state hypothesized to be the core feature of TM's effects (Travis and Pearson, 2000; Travis and Shear, 2010; Mason et al., 1997).

Future randomized controlled trials will be required to confirm that TM practice causally reduces CTRA activity, but it is plausible to expect such effects given TM's capacity to alter neurobiological processes related to the sympathetic nervous system activity that mediates CTRA gene regulation (Cole, 2019). For example, an early study reported that lymphocytes from long-term TM practitioners showed lower β_2-adrenergic activity compared to controls (Mills et al., 1990). Also, prospective randomized research designs have linked TM practice to alterations in HPA axis activity and other neuroendocrine processes both during and outside the practice (see (MacLean et al., 1997; Walton et al., 2002) for review). Other well controlled, short term studies have found beneficial effects of TM in the treatment of posttraumatic stress disorder (Nidich et al., 2018) and cardiovascular disease (Schneider et al., 2012, 2019; Paul-Labrador et al., 2006; Castillo-Richmond et al., 2000; Walton et al., 2002). It may be noteworthy that the only adversities known to have been experienced by participants in the present study were ordinary levels of psychosocial stress and advancing age (mean age, 64 years). Prior randomized, controlled trials of TM have reported relief from both psychosocial stress in students (MacLean et al., 1997) and aging-related measures in octogenarians (Alexander et al., 1989).

Despite the clear presence of a CTRA-characteristic transcriptome signature in this sample of long-term TM practitioners *vs*. controls, several limitations need to be considered in interpreting these results. These data come from a hypothesis-driven secondary analysis of a small (n = 12) observational study conducted at a single time-point (i.e., no pre-TM baseline) and at a single site. Thus, the generalizability and robustness of these findings remain to be determined in future experimental research with larger and more diverse samples. Causal conclusions cannot be definitively drawn from the comparative observational design of the study. No health outcomes were examined in this study, so the health significance of the CTRA differences observed here remains to be determined in future research. The present study did not involve any active comparison condition (e.g., comparison with another form of meditation such as mindfulness). Consequently, also remaining to be determined is how effects of TM might resemble or be distinct from those of other meditative practices. Given the distinctive features of TM (Travis and Shear, 2010), some differences might be anticipated, but their nature remains to be empirically defined in future research.

One additional concern for interpreting the present results and for future experiments is the effect of social isolation and social support. Key evidence leading to the original discovery of the CTRA pattern emerged while studying individuals who consistently reported feeling 'lonely' and 'distant from others' (Cole, 2013). An individual's participation in an intervention program may entail more (or in some cases, less) social interaction, with corresponding effects on the CTRA pattern (Cole et al., 2021). Possible systematic differences in social interaction were not considered in the present study. Future research should attempt to control for social interactions as well as other potentially important behavioral variables specific to the intervention. For long-term studies such as this one, an appropriate active control group is difficult to imagine, but career writers involved daily in inward mental activity might be an option. Finally, this hypothesis-targeted analysis focused specifically on the CTRA. Other transcriptional effects associated with TM likely exist in this sample, as suggested by the exploratory findings (Wenuganen et al., 2021).

Interpretive limitations notwithstanding, the present analyses demonstrate that a sample of TM practitioners exhibited reduced CTRA activity compared to demographically matched controls. In combination with results from previous studies of short-term meditation practice, this finding supports the likely value of further studies designed to evaluate causality, specificity, and possible connections to health and aging.

Declaration of competing interest

None.

Data availability

Data used are publicly available at the address mentioned in Materials and Methods.

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