Differential DNA methylation in experienced meditators after an intensive day of mindfulness-based practice: Implications for immune-related pathways


Abstract

The human methylome is dynamically influenced by psychological stress. However, its responsiveness to stress management remains underexplored. Meditation practice has been shown to significantly reduce stress levels, among other beneficial neurophysiological outcomes. Here, we evaluated the impact of an intensive meditation practice (t2−t1 = 8 h) on the methylome of peripheral blood mononuclear cells in experienced meditators (n = 17). In parallel, we assessed the influence of a day of leisure activities in the same environment on the methylome of matched control subjects with no meditation experience (n = 17). DNA methylation profiles were analyzed using the Illumina 450 K beadchip array. We fitted for each methylation site a linear model for multi-level experiments which adjusts the variation between t1 and t2 for baseline differences. No significant baseline differences in methylation profiles was detected between groups. In the meditation group, we identified 61 differentially methylated sites (DMS) after the intervention. These DMS were enriched in genes mostly associated with immune cell metabolism and aging and in binding sites for several transcription factors involved in immune response and inflammation, among other functions. In the control group, no significant change in methylation level was observed after the day of leisure activities. These results suggest that a short meditation intervention in trained subjects may rapidly influence the epigenome at sites of potential relevance for immune function and provide a better understanding of the dynamics of the human methylome over short time windows.

1. Introduction

There is growing evidence that the human epigenome is influenced by psychological stress. In particular, stressful life events have been shown to lead to long-lasting methylation at the glucocorticoid receptor gene and other methylation marks across the genome (Burns et al., 2018; McGowan and Roth, 2015). Such epigenetic effects may mediate the embodiment of stressful life events and contribute to their physiological and behavioral outcomes, such as the persistent cognitive alterations, activation of the HPA axis and increased risk for psycho-pathology and chronic diseases (Brown et al., 2019; McEwen, 2017).

Fewer studies have investigated the extent to which stress management shapes the human epigenome, and may counterbalance these deleterious stress-induced epigenetic effects. Meditation, a family of practices based on attentional and emotional regulation (Lutz et al., 2008), has been shown to significantly reduce stress (Pascoe et al., 2017), among other beneficial outcomes at the emotional and cognitive levels (Barnhofer, 2019; Dahl et al., 2015; Klimecki et al., 2019; Luders...
2. Results

In this context, cross-sectional studies have shown that blood cells from long term meditators exhibit a trajectory of epigenetic ageing different from age-matched meditation-naïve controls, with a slowdown of the epigenetic clock as the number of years of practice increases (Chaix et al., 2017), and epigenetic changes in pathways related to common diseases and inflammatory signaling (García-Campayo et al., 2018). In addition, a longitudinal study in meditation trained subjects showed that a day of intensive mindfulness meditation induced a decrease in the expression level of histone deacetylases genes, as well as significant changes in histone acetylation levels, when compared to an active control group of meditation-naïve subjects engaged in leisure activities in the same environment (Kaliman et al., 2014). Consistent with the emerging anti-inflammatory role of HDAC inhibitors (HDACi) (Galta et al., 2019), the decrease in HDAC gene expression in the meditation group was concomitant with a significant downregulation of proinflammatory genes Receptor Interacting serine/threonine Kinase 2 (RIPK2) and Cyclooxygenase-2 (COX2), which are regulated by HDACi in diverse cell systems (Tong et al., 2004; Roger et al., 2019).

Because histone deacetylases and histone modifications play a key role in epigenetic regulation, these findings raise the possibility that the meditative practice may rapidly modulate the human epigenome and influence clinically relevant signaling pathways. To test this hypothesis, we performed the first methylene-wide longitudinal study in peripheral blood mononuclear cells (PBMCs) from the same group of meditators and controls analyzed in our previous study (Kaliman et al., 2014), using high-throughput DNA methylation data.

2.1. Identification and localization of DMS

We obtained paired PBMCs samples from 18 meditators and 20 controls, before (t₁) and after (t₂) 8 h of a mindfulness meditation session for the meditators, and 8 h of leisure activities in the same environment for the meditation-naïve controls. DNA methylation profiles at 485,512 sites across the genome were analyzed for all samples using the Illumina 450 K beadchip array. After quality filtering, we retained a dataset comprising 414,717 methylation sites for 17 meditators and 17 controls individuals at t₁ and t₂. The normalization procedure to remove technical noise and to maximize signal detection is described in detail in Supplementary note 1. To correct methylation values for heterogeneity in blood cell types proportions, cell counts were estimated from methylation levels for each sample using a validated regression calibration algorithm (Accomando et al., 2014; Jaffe and Irizarry, 2014). No significant difference in blood cell types proportions was observed between t₁ and t₂, nor between controls and meditators (Supplementary Fig. 1, t test p-values greater than 0.05 for both cells). Cell types proportions were taken into account in all subsequent analyses, along with gender and age.

2.2. Functional analysis of meditation-sensitive DMS

We tested whether specific gene ontology categories or KEGG pathways were significantly enriched in meditation-sensitive DMS. Four KEGG pathways exhibited a significant enrichment in meditation-sensitive DMS after multiple testing correction (Table 1): two of these categories were related to fatty acid metabolism, comprising genes ACADM, CPT1A and HSD17B4. Other categories significantly enriched were related to RNA transport (with genes SAP18, EIF1B, NCBP2), and to the Fanconi anemia pathway (with genes APITD1 and ERCC1), which is involved in the preservation of the genome stability. The top 5 GO biological processes, were linked to betaine metabolism, fatty acid metabolism and immunity, although none of them showed significant enrichment in meditation-sensitive DMS after multiple testing correction (Supplementary Table 5).

To investigate further the biological functions of genes associated to meditation-sensitive DMS, we performed a gene-gene interacting network using the STRING database, which integrates known and predicted protein–protein interactions, including physical (direct) and functional (indirect) associations (Supplementary Fig. 3). This analysis supported results from the KEGG pathways analysis. In particular, ACADM, CPT1A and HSD17B4 emerged as a functional cluster involved in the beta-oxidation pathway within fatty acid metabolism. The largest cluster comprised APITD1, ERCC1, PHF21A, SAP18, HNRNPH1, NCBP2, EIF4E3 and GPR27 genes which are involved in DNA repair (APITD1...
Fig. 1. Heatmap of meditation-sensitive differentially methylated sites (DMS). 61 sites showed methylation levels either significantly increased or decreased (after multiple testing correction) by at least 3% in the meditator group after the meditation intervention (t2) in comparison to before the intervention (t1). Each column is one individual (t1 on the left and t2 on the right), each line is one meditation-sensitive DMS. Sites with the largest increase in methylation are represented at the top (e.g. the gene TBKBP1 exhibited the largest increase). The color indicates the relative methylation level, from low (green) to high (red). CpG ID: Illumina CpG ID, Diff: difference in methylation level between t2 and t1, P-val: FDR adjusted P-value, TSS200: proximal promoter (less than 200 bp upstream from the transcription start site), TSS1500: distal promoter (from 1,500 to 200 bp upstream from the transcription start site). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
and ERCC1 were associated to the Fanconi anemia pathway in the KEGG analysis), in chromatin remodeling and in RNA metabolism and protein translation (Costello et al., 2017; Volpon et al., 2013). STRING analysis also revealed 3 additional gene-gene interactions: NRCAM and SPTBN1, involved in axonal growth and neurotransmission (Amor et al., 2017); DAXX and TERT, involved in telomerase regulation and telomeres maintenance (Tang et al., 2015); ITGA6 and MCAM, involved in cell adhesion (Shirali et al., 2018).

2.3. Motif enrichment analysis

We performed a motif enrichment analysis (McLeay and Bailey, 2010), considering a flanking sequence of 150 bp (± 75 bp) around each meditation-sensitive DMS. Five motifs were found significantly enriched, corresponding to binding sites for KLF15, EGR1, EGR2, SP3 and SP4 transcription factors which are involved in immune response and inflammation as well as other biological processes. Table 2 presents the enriched motifs and corresponding transcription factors as well as references relevant in the context of immunity and inflammation. Notably, KLF15 (Krüppel-like factor 15) regulates vascular inflammation through the interaction with NF-κB, and its expression is directly induced by glucocorticoids (Mcconnell and Yang, 2010; Lu et al., 2013). SP3 (Specificity protein 3) controls the expression of inflammation-related molecules such as the anti-inflammatory cytokine IL-10 and the pro-inflammatory molecule COX-2 (Lee et al., 2006; Tone et al., 2019). When considering in this analysis a longer flanking sequence around each DMS (500 bp), the same 5 motifs were significantly enriched, along with 29 other motifs (the top 10 enriched motifs and their biological activity are summarized in Supplementary Table 6).

3. Discussion

While it has been reported by many studies that the human methylome is dynamically influenced by psychological stress, its responsiveness to stress reduction remains poorly explored. Here, we performed a methylome-wide scan to detect differential methylation in response to an intense 8 h meditation practice in subjects with long-term meditation training. We found that the day long intensive practice of mindfulness meditation impacted the methylation profile of the participants at 61 CpG sites (over 3% change in methylation level and p-value below 0.05 after multiple testing correction). No significant change in methylation profiles was seen in the control group in response to the leisure day. In addition, there was no significant basal difference between the meditators and the controls in terms of methylation profiles. This corroborates the previously reported absence of basal difference between the two groups in terms of transcriptomic profiles (Kaliman et al., 2014).

We analyzed the biological pathways associated with the 61 meditation-sensitive DMS through KEGG pathway enrichment analysis (Table 1), STRING analysis (Supplementary Fig. 3) and Motif enrichment analysis (Table 2). A previous transcriptome and protein level analysis performed on the same samples highlighted that this intervention led to a decrease in HDAC gene expression and to a significant downregulation of proinflammatory genes (Kaliman et al., 2014).
The pathway most significantly enriched in mediation-sensitive DMS was fatty acid metabolism, with a differential methylation level of 3% or more. This pathway is involved in the metabolism of fatty acids, which are essential components of cell membranes and are used as energy sources. Fatty acid metabolism is regulated by a variety of factors, including hormones and nutrients, and is involved in the development of various diseases, such as obesity and diabetes.

Consistent with previous findings, here we detected differential methylation in biological pathways of relevance for the inflammatory and immune systems after the meditation intervention. In particular, the 61 meditation-sensitive DMS were significantly enriched in binding sites for five transcription factors (KLF15, EGR1, EGR2, SP3, SP4) involved in immunity and inflammation among other biological processes (McConnell and Yang, 2010; Lu et al., 2013; Lee et al., 2006; Tone et al., 2019; Sheehan et al., 2019; Li et al., 2012; Miao et al., 2017). The enrichment in the binding site SP3 (Specificity protein 3) is particularly interesting for our study as this transcription factor controls the expression of the pro-inflammatory molecule cyclooxygenase-2 COX2 (Lee et al., 2006; Tone et al., 2019). Indeed, a previous report on the same samples analyzed here has shown that the daylong meditation intervention significantly decreased the gene and the protein expression of COX2 (Kallman et al., 2014). As we did not detect significant changes in the methylation level of COX2 gene, these data suggest that the meditation practice may modulate the expression of this gene through changes in the activity of specific regulatory transcription factors, rather than through methylation changes. This corroborates a recent study suggesting that changes in transcription levels may precede changes in methylation level (Pacis et al., 2019). In addition, the greatest methylation change (in terms of magnitude) in response to the intervention localized in the TLRBP1 gene which is involved in the TNF-α/ NF-κB pathway (Fig. 1). This pathway is activated in blood cells under conditions of acute and chronic psychological stress (Pace et al., 2006; Miller et al., 2008; Miller et al., 2009). Notably, a decrease in NF-κB activity seems to be a consistent genomic fingerprint of mind–body therapies, that may underlie the potential anti-inflammatory effects of these practices (Black and Slavich, 2016; Bucy, 2017). At least two other methylation sensitive DMS are located in genes previously associated with immune and inflammatory pathways: TNFSF13B and PRF1, whose expression and methylation level in blood cells was previously shown to respond to psychological stress exposure, and in particular to the Trier Social Stress Test (TSST) (Falkenberg et al., 2013). In addition, two intergenic meditation-sensitive DMS, cg12989851 and cg12496710, were previously shown to have their methylation level in blood associated with inflammatory disorders (Imgenberg-Kreuz et al., 2016; Liu et al., 2013).

Consistent with the role of the ANS and the HPA axis in the regulation of energy balance (Peckett et al., 2011), lipid metabolism was the pathway most significantly enriched according to the KEGG analysis, with genes ACADM, CPT1A and HSD17B4. These genes also emerged as a cluster in the STRING analysis. Our findings suggest that this metabolic pathway might have been modulated by the stress reducing effects associated with meditation (Pasco et al., 2017). Immune cells use lipids as a source of energy, as any other cell, by degrading fatty acids in a process termed beta-oxidation. However, lipid metabolism is also currently viewed as a central switch regulating T cell fate decisions. Fatty acid oxidation seems to guide specific T cell fates and functions including the induction of CD4 + regulatory T and CD8 + memory T cells (Lochner et al., 2015). Here we analyzed PBMCs, which are composed of lymphocytes (T cells, B cells, and NK cells) in the range of 70–90 % (K.C.R., 2015). Previous studies have reported significant increases in antibody titers to influenza vaccine (Davidson et al., 2003) and effects on CD4 + T lymphocytes in HIV-1 infected adults (Creswell et al., 2009), in response to MBIs. Based on the data presented here, we hypothesize that immune function improvement by stress reduction strategies could be mediated at least in part by methylation changes in specific fatty acid metabolism genes. This hypothesis warrants further investigation.

The pathway most consistently differentially methylated (according to the KEGG, STRING and motif enrichment analyses) after the meditation intervention involves DNA repair and response to DNA damage. More precisely, the Fanconi anemia pathway, that preserves genome stability (Moldovan and D’Andrea, 2009), was significantly enriched in
Supporting these findings, the expression levels of MCAM and ITGA6, which are involved in angiogenesis, were found to be higher in long-term meditators compared to controls, as well as a slowdown of their epigenetic clock with the number of years of meditation practice (Chaix et al., 2017). Another KEGG pathway significantly enriched among the 61 meditation-sensitive DMS was related to RNA transport, and includes genes SAP18, EIF1B, NCBP2. We also observed that the largest cluster that emerged in the STRING analysis comprises NCBP2, EIF4E3 and GPR27 genes that are involved in RNA metabolism and protein translation. In addition, this largest cluster in the STRING analysis is also enriched in genes involved in chromatin remodeling and in epigenetic regulation, in particular PHF21A (Garay et al., 2016) and SAP18 (Zhang et al., 1997) that recruit histone deacetylase (HDAC)-containing complexes. This suggests that chromatin recruitment and the activity of histone deacetylases might be sensitive to meditation practice. Taking into account the role of histone acetylation in inflammation (Garay et al., 2016) and the anti-inflammatory cytokine IL-10 through the interaction with NF-κB, and its expression is directly induced by glucocorticoids (Mcconnell and Yang, 2010; Lu et al., 2013).

Table 2

<table>
<thead>
<tr>
<th>Logo Rank</th>
<th>Symbol</th>
<th>adj. P-value</th>
<th>E-value</th>
<th>N' of DMS</th>
<th>Full name; function</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>KLF15</td>
<td>4.42e-6</td>
<td>1.78e-3</td>
<td>16</td>
<td>Krüppel-like factor 15; involved in cellular glucose homeostasis, stress response and inflammation. KLF15 regulates vascular inflammation through the interaction with NF-κB, and its expression is directly induced by glucocorticoids (Mcconnell and Yang, 2010; Lu et al., 2013).</td>
</tr>
<tr>
<td>2</td>
<td>EGR1</td>
<td>7.82e-6</td>
<td>3.14e-3</td>
<td>23</td>
<td>Early Growth Response gene 1; involved in many biological processes, such as response to DNA damage, immunity, and inflammatory responses</td>
</tr>
<tr>
<td>3</td>
<td>SP3</td>
<td>8.91e-6</td>
<td>3.58e-3</td>
<td>17</td>
<td>Specificity protein 3; involved in response to DNA damage, immunity, hematopoiesis, and controls the expression of inflammation-related molecules such as the anti-inflammatory cytokine IL-10 and the pro-inflammatory molecule COX-2 (Lee et al., 2006; Tone et al., 2019)</td>
</tr>
<tr>
<td>4</td>
<td>SP4</td>
<td>1.59e-5</td>
<td>6.39e-3</td>
<td>16</td>
<td>Specificity protein 4; involved in inflammatory and neuropathic persistent pain states (Sheshan et al., 2019) and in dendrite patterning and neurotransmission (Lä et al., 2012)</td>
</tr>
<tr>
<td>5</td>
<td>EGR2</td>
<td>4.32e-5</td>
<td>1.74e-2</td>
<td>22</td>
<td>Early Growth Response gene 2; involved in immunity and inflammatory processes (Miao et al., 2017; Paci et al., 2019), as well as various neuropathies.</td>
</tr>
</tbody>
</table>

the KEGG analysis, with genes ERCC1 and APITD1. This pathway was also highlighted in the largest cluster that emerged in STRING analysis. Moreover, about a third of the 61 meditation-associated DMS are located in and nearby binding sites for transcription factors EGR1 and SP3 that play a role not only in immune function but also in the response to DNA damage. This observation of differential methylation in the DNA damage response pathway may be related to the stress reducing effects of the meditation practice, as previous reports suggest an association between exposure to psychological stress and DNA damage (Flint et al., 2007; Gidron et al., 2006). Moreover, our study identified meditation-induced DMS associated with cell aging. Strikingly, one of them localized in the gene body of TERT (telomerase reverse transcriptase), that codes for a subunit of the telomerase enzyme. This enzyme elongates telomeres, which are nucleoproteins complexes protecting the ends of chromosomes from instability and degradation. A cluster linking TERT to DAXX gene, shown to be involved in telomere maintenance, also emerged in the STRING analysis. Greater overall telomere attrition predicts mortality and aging-related diseases and both telomeres length and telomerase activity are sensitive to psychological stress (Blackburn et al., 2015). On the other hand, there is emerging evidence that MBIs may modulate telomerase activity, and possibly, increase telomere length (Conklin et al., 2019). These results suggest that meditation practice may elicit epigenetic changes that are associated with telomere biology, and more generally biological aging. Our findings showing enrichment in DMS associated with DNA damage and cell aging are particularly interesting in the context of PBMCs. Indeed, increased DNA damage and telomere attrition in the immune system lead to the accumulation of genomically damaged and senescent cells (Keenan and Allan, 2019) and cellular senescence seems to be associated with low grade chronic inflammation through the immune cell acquisition of the senescence-associated secretory phenotype (López-Otín et al., 2013; Sikora et al., 2011). Taken together, these data suggest that MBIs may help prevent or delay immune system aging at least in part through epigenetic regulation of DNA repair mechanisms and telomere biology. These findings corroborate our recent observation of different epigenetic aging trajectories in PBMCs between long term meditators and controls, as well as a slowdown of their epigenetic clock with the number of years of meditation practice (Chaix et al., 2017).
sensitive DMs correspond to binding sites for transcription factor GPR19 et al., 2018) associated with addiction related behavior. PACSIN1 has been previously reported as potential blood biomarkers of mental health: a number of genes that contain meditation-sensitive DMS have been previously reported long lasting effects of this practice remains to be this daylong meditative practice in experienced practitioners and the

Most of the 61 meditation-sensitive sites exhibited an increase in methylation level and were located upstream of genes (promoters and first exons) and in CpG islands. DNA methylation generally plays a repressing role on gene expression, especially when located at CpGs upstream of genes. Consequently, some of the methylation changes we report here may be associated with a decreased gene expression. We interpret this skewed landscape of increased methylation at sites located upstream of genes as a methodological lack of sensitivity to detect decreases in methylation at CpG islands which are known to depict low basal methylation levels (Hernando-Herraez et al., 2015; Jones and Takai, 2001). Similarly, our study did not detect significant changes in methylation profiles in the control group after the leisure day. The lack of participant randomization due to the fact that we compared meditation experts with unexperienced subjects, may, at least in part, explain this result. Moreover, while the meditation group was instructed to follow the same mental-training practices throughout the intervention, participants in the control group could voluntarily choose between a variety of activities (i.e. resting, reading, watching documentaries or playing computer games). Such heterogeneity of leisure activities may have introduced a variability decreasing the statistical power to detect specific changes on DNA methylation in the control group. These observations further highlight the need to design future studies with active control interventions that are structurally comparable to the meditation interventions (Davidson and Kaszniaik, 2015). In addition, in the future, it will be important to determine if a one-day intervention in otherwise naïve participants would produce any of the effects we observed in the current study performed in long term meditators.

To conclude, using stringent criteria for DMS identification, our study shows that a short meditation intervention in trained subjects rapidly influenced the methylome at sites of potential clinical relevance, related to the transcriptional regulation of the inflammation response, immune cell metabolism, DNA repair, cell aging, RNA metabolism, protein translation, cell adhesion and neurotransmission. Whereas methylation marks are usually considered to be relatively stable, our observations strengthen a growing body of research in humans and murine models reporting fast methylation changes, in particular in response to acute stress (Falkenberg et al., 2013; Mifsud et al., 2017; Saunderson et al., 2016; Rodrigues et al., 2015; Unternaehrer et al., 2012). Altogether, these studies depict the complexity of methylome dynamics, combining both stable and environmentally labile marks. The relationship between the fast epigenetic changes elicited by this daylong meditative practice in experienced practitioners and the previously reported long lasting effects of this practice remains to be investigated. Future randomized controlled studies with larger sample sizes, active control groups and long-term follow-ups are required to validate the findings of this initial study and to explore their health-related potential.

4. Material and methods

4.1. Participants and interventions.

Long-term meditators (n = 19) and meditation-naïve controls (n = 21) were studied. The demographic characteristics of the groups and interventions are described more extensively in (Kaliman et al., 2014). Both groups showed similar distributions of age (controls: 50.38 ± 8.96; meditators: 49.89 ± 11.18), gender (controls: 12F and 9 M; meditators: 11 F. 8 M), race (controls: 95.23% Caucasian; meditators: 84.21% Caucasian) and body-mass index (controls: 23.55 ± 3.76; meditators: 24.16 ± 3.51). Their peripheral blood mononuclear cells (PBMC) were obtained at 8 am (Time 1) and at 4 pm (Time 2), respectively before and after an intensive day of mindfulness meditation for the meditators, and a day of leisure activities in the same environment for the controls. The UW-Madison Health Sciences Internal Review Board approved this study and all participants provided written informed consent. The criteria for inclusion in the meditators group were i) a daily meditation practice spanning a minimum of 3 years, ii) at least 30 min of daily sitting meditation and iii) a minimum of 3 intensive retreats lasting 5 or more days. All experienced meditators practiced both standard mindfulness-related meditations (e.g. Vipassana and concentration meditations) and compassion-related meditations. The controls had no prior meditation experience. The interventions for the meditators and the control group were matched in terms of physical activity. The meditation intervention largely overlapped in terms of contents with the day-long session of the Mindfulness-Based Stress Reduction program (MBSR), that is routinely used in North-America hospitals (Kabat-Zinn, 1982). The controls were engaged in intentional activities, such as reading, watching documentaries or playing computer games, and walking.

4.2. Isolation of peripheral blood mononuclear cells (PBMC)

Blood samples were obtained from each participant and PBMCs were isolated, as described in Kaliman et al. (Kaliman et al., 2014). DNA was isolated using QiAamp DNA Blood Mini Kit, and stored at −80 °C until processing. Samples from two participants from the original study could not be analyzed due to insufficient DNA quality.

4.3. Genome-wide DNA methylation profiling

We used the Infinium HumanMethylation450 beadchip array (Bibikova et al., 2011) to examine methylation levels at 485,512 sites for 79 samples, including 20 pre-intervention (t1) controls, 18 post-intervention (t2) meditators, 18 pre-intervention (t1) meditators, 18 post-intervention (t2) meditators, as well as 3 technical replicates. DNA methylation data from all participants were generated at the same time, in a single batch. DNA samples from both groups were randomized across the arrays.

4.4. Sample filtering, probe filtering and data normalization procedure

Sample filtering, probe filtering and data normalization procedure are presented in Supplementary note 1.

4.5. Accounting for heterogeneity in cell subtypes

The DNA samples have been extracted from a mixture of cell types (PBMC). Consequently, it is crucial to take into account the heterogeneity in cell types proportions across samples in our analyses, since this heterogeneity may introduce a variation in the data (Houseman et al., 2012). To estimate the relative proportions of CD8 + T cells, CD4 + T cells, NK cells, B cells, and monocytes for each participant, we used the estimate Cell Counts function from the R Bioconductor package Minfi (Aryee et al., 2014), a regression calibration algorithm for deconvoluting heterogeneous tissues (Jaffe and Irazarry, 2014; Ritchie et al., 2015). The estimated cell counts were rescaled to 1 and were taken into account as covariates in all subsequent analyses. The probes that were used to predict cell counts proportions were removed from the data set for subsequent analyses, yielding a final set of 414,717 probes.
4.6. Determination of differentially methylated sites (DMS) after a day of intensive meditative practice

Sites differentially methylated between meditators and controls and between t1 and t2 were identified statistically by fitting at each site a linear mixed model (M = values ~ Mt1 + Mt2 + Ct1 + Ct2 + sex + age + cell type proportions + error), with the R bioconductor limma package (Benjamini and Hochberg, 1995). Mt1, Mt2, Ct1, and Ct2 are binary variables indicating whether data correspond to meditator (M), control (C), pre (t1) or post (t2) intervention. Individuals were treated as random effects. This model is specifically designed for multi-level experiments and allows comparing simultaneously two groups (here meditators and controls) and comparing paired samples (here two samples from the same individual, in t1 and t2). Crucially, it adjusts the detected variation between t1 and t2 for baseline differences in methylation profiles. Note that the paired test used to compare data within subjects is expected to be more powerful than the non-paired test used to compare data between subjects. We used a contrast matrix to identify sites with signals of differential methylation between meditators and controls and between t1 and t2 in meditators and in controls. Sites with a FDR adjusted P-value (M. Ashburner and Consortium, 2000) below 0.05 and a difference in methylation level between t1 and t2 of at least 3% were considered to be differentially methylated (DMS). This difference in methylation level was computed on β-values adjusted for age, gender and cell type proportions.

4.7. Genomic features of differentially methylated sites

To characterize the spatio-functional localization of DMS, we assessed whether particular genomic regions were enriched in meditative-sensing DMS. For each site, we used the Illumina HumanMeth450 annotation table to identify DMS located within genomic regions, as well as their precise genomic location using the UCSCRefGene Group column: into a distal promoter (TSS1500, from 1,500 to 200 bp upstream from the transcription start site), a proximal promoter (TSS200, less than 200 bp upstream from the transcription start site), a 5’UTR, a first exon, a gene body or a 3’UTR. Then, we used the Relation_to_Island column to identify the DMS located into CpG islands, shores (from 0 to 2 kb from islands), shelves (from 2 to 4 kb from islands) and open seas (more than 4 kb from islands). We assessed the enrichment of these regions among the identified DMS through the computation of an Odd-Ratio, defined as

\[ OR = \frac{P(R|DMS)}{P(R|notDMS)} \cdot \frac{P(notR|notDMS)}{P(notR|DMS)} \]

with R being ‘in the region’. Significance of enrichment was tested for each region (over all meditators or all controls) using Chi-square test.

4.8. Gene ontology (GO) and KEGG analysis

We performed an analysis of the over-representation of gene ontology (GO) categories (Phipson et al., 2015) or KEGG pathways (Kyoto Encyclopedia of Genes and Genomes) among differentially methylated sites. We used the gometh function from the R bioconductor package to characterize the spatio-functional localization of DMS, we assessed whether particular genomic regions were enriched in meditative-sensing DMS. For each site, we used the Illumina HumanMeth450 annotation table to identify DMS located within genomic regions, as well as their precise genomic location using the UCSCRefGene Group column: into a distal promoter (TSS1500, from 1,500 to 200 bp upstream from the transcription start site), a proximal promoter (TSS200, less than 200 bp upstream from the transcription start site), a 5’UTR, a first exon, a gene body or a 3’UTR. Then, we used the Relation_to_Island column to identify the DMS located into CpG islands, shores (from 0 to 2 kb from islands), shelves (from 2 to 4 kb from islands) and open seas (more than 4 kb from islands). We assessed the enrichment of these regions among the identified DMS through the computation of an Odd-Ratio, defined as

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with R being ‘in the region’. Significance of enrichment was tested for each region (over all meditators or all controls) using Chi-square test.

4.9. Protein-protein association representation by STRING database analysis

We constructed the protein–protein interaction network based on the list of DMS-associated genes found in this study using the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) https://string-db.org.

4.10. Transcription factor motif discovery

The DNA sequences flanking the DMS found in the study were used to identify enriched motifs using the AME suite package (McLeay and Bailey, 2010), a part of the MEME Suite online platform. An E-value cut-off of 0.05 was considered to identify significantly enriched motifs. We ran two independent analyses, considering flanking sequences around each DMS of either 150 (± 75) or 500 (± 250) bp length.

Acknowledgements

This work was supported by CNRS PESP INEE 2015 (RC), NCCAM (NIH) (PO1-AT004952) (RJD and AL), Fetzer Institute, John Templeton Foundation, anonymous donor (RJD), LABEX CORTEX ANR-11-LABX-0042, Université de Lyon ANR-11-IDEX-0007 and ERC-Consolidator 617739-BRAINANDMINDFULNESS (AL). We thank Hélène Quach, Johanna Lepeule, Lucas Husquin, Hervé Perdry, Alice Urvoy and Barry Kerzin for helpful discussions.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbi.2019.11.003.

References


