Differential Gene Expression after Emotional Freedom Techniques (EFT) Treatment: A Novel Pilot Protocol for Salivary mRNA Assessment

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Abstract

Biopsychology is a rapidly expanding field of study since the completion of the Human Genome Project in 2003. There is little data measuring the effect of psychotherapeutic interventions on gene expression, due to the technical, logistical, and financial requirements of analysis. Being able to measure easily the effects of therapeutic experiences can validate the benefits of intervention. In order to test the feasibility of gene expression testing in a private practice setting, this study compared messenger ribonucleic acid (mRNA) and gene expression before and after psychotherapy and a control condition. With four non-clinical adult participants, it piloted a novel methodology using saliva stored at room temperature. A preliminary test of the interleukin-8 (IL8) gene in both blood and saliva was performed in order to determine equivalency in the two biofluids; convergent validity was found. Following saliva test validation, a broad, genome-wide analysis was performed to detect differential gene expression in samples collected before and after treatment with Emotional Freedom Techniques (EFT), an evidence-based practice combining acupressure and cognitive exposure. The control treatment was non-therapeutic social interaction. To establish a baseline, participants received the control first, followed a week later by EFT. Analysis of samples was performed at three time points: immediately before treatment, immediately after, and 24 hours later. Differential expression between EFT and control was found in numerous genes implicated in overall health ($p < 0.05$). Further, the differentially expressed genes in this study were shown to be linked to immunity, pro or anti-inflammatory, as well as neuronal processes in the brain. Ten of the 72 differentially expressed genes are identified as promising targets for downstream research. The data show promise for the future use of salivary samples to determine the effects of therapy; this pilot protocol also illustrated the challenges and limitations of novel technologies employed in biopsychology.

Keywords: epigenetics, DNA, mRNA, gene expression, protein synthesis, brain plasticity, neurogenesis, biopsychology

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Psychology is a broad field with many procedures and schools of thought regarding the treatment of mental and emotional problems. As the field broadens with ever-evolving eclecticism and fine-tuning of psychotherapeutic techniques and modalities, more questions arise that pertain to the biological mechanisms behind client recoveries and transformations following treatment and self-maintenance. It is theorized that any novel experience, including experiential psychotherapeutic interventions, can impact gene expression in humans, resulting

in brain changes. This phenomenon is known as neurogenesis or brain plasticity (Siegel, 1995; Kandel, 1998, 2001; Montag-Sallaz, Welzl, Kuhl, Montag, & Schachner, 1999; Rossi, 2002; Rutishauser, Mamelak, & Schuman, 2006). It has been argued that effective psychotherapy may be viewed as an epigenetic intervention, regulating stress genes such as those that code for cortisol and epinephrine, as well as regulating the autonomic nervous system (Feinstein & Church, 2010; Church 2013c).

The science behind the merging fields of biology and psychology has proliferated over the last decade with researchers in both fields integrating aspects of the other (Rossi, 2002; Rossi, Rossi, Yount, Cozzolino, & Iannotti, 2006; Siegel, 2012). Technologies have emerged in the fields of functional magnetic resonance imaging (fMRI; Petrella, Mattay, & Doraiswamy, 2008), endocrinology (Yehuda et al., 2009), and molecular biology (Yount, 2013) that permit the experimental testing of the effect that psychotherapy has on neurogenesis (Eriksson et al., 1998; Ackerman, Martino, Heyman, Moyna, & Rabin, 1998; Montag-Sallaz et al., 1998; Ramanan et al., 2005; Xiang et al., 2008; Boyke, Driemeyer, Gaser, Büchel, & May, 2008). Recent gene expression research has enabled investigators to study the effects of experiences such as psychotherapeutic interventions. The notion that environment and experience change the brain's neurological wiring has evolved from a hypothesis into an empirically demonstrated reality (Anderson et al., 2004; Erk et al., 2010; Hölzel et al., 2011).

Psychotherapy and Neuroplasticity

Psychotherapeutic modalities are broadly effective, with no one method showing clear superiority over others (Wampold, Mondin, Moody, Stich, Benson, & Ahn, 1997; Ahn & Wampold, 2001). Psychotherapy is also efficacious for physical conditions, with a great deal of evidence supporting the link between mental and physical health (Alexander, Arnkoff, & Glass, 2010; Church, 2013c). The American Psychological Association (APA) recognizes the benefits and effectiveness of psychotherapy, and suggests that psychotherapy should continue to be included within the primary health care system. Though evidence supports equivalency and comparability of psychotherapeutic interventions, experiential and somatic therapies have been shown to yield improvements in much shorter treatment time frames (Greenberg & Watson, 1998; Karatzias et al., 2011; de Roos et al., 2011; Church, 2013a, 2013c). These interventions typically include techniques that induce the relaxation response (RR) to lower emotional distress, anxiety, or insomnia. RR meditation, defined as a mind-body intervention, is known to offset the physiological effects of stress (Benson & Klipper, 1975).

Brain plasticity, or neurogenesis, is the lifelong ability of the brain to change, grow, and reorganize neural pathways based on new experiences and even injury (Eriksson et al., 1998; Rossi, 2002). Genetic processes have been shown to result in neuronal growth in the brain by increasing the number of synapses between neurons (Eriksson et al., 1998; Kandel, 1998, 2001; Neville & Bavelier, 2000). These are the processes responsible for neuronal brain growth by way of genetic processes, also known as brain plasticity. Plasticity may be triggered by adverse life experiences, such as trauma, loss, and injury. Plasticity may also be triggered by positive experiences such as novelty, learning, and psychotherapeutic interventions. Therapies that employ this effect may therefore be regarded as epigenetic interventions (Church, 2013c).

Experiences trigger protein synthesis mediated by messenger ribonucleic acid (mRNA), resulting in a cascade of physiological, neuronal, and structural changes (Strachan & Read, 1999). Combining the study of psychotherapy and the processes of neurogenesis is referred to as Interpersonal Neurobiology (Siegel, 2012). Empirical investigation in this area of study has been challenged due to the lack of a noninvasive method of sample collection. The validation of a biofluid collection protocol would allow the measurement of gene expression and the exploration of the effects of psychotherapy as an epigenetic intervention.

Epigenetics and Neuroplasticity

Genes are the mechanisms by which living organisms inherit features from their ancestors. The genotype is the genetic makeup of a cell, an organism, or an individual. The cell interprets the genetic code stored in DNA when the gene is expressed, and the properties of that expression give rise to the organism's phenotype and observable characteristics, including behavior.

Gene expression can be influenced by environment and experience resulting in phenotypic changes such as neurogenesis. mRNA is an

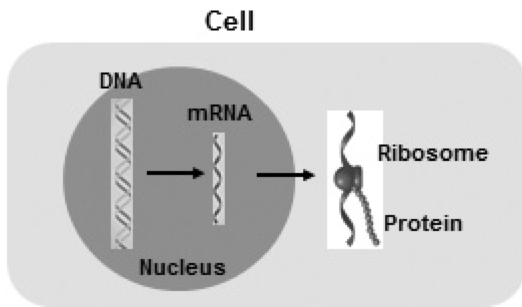


Figure 1. The blueprint in DNA for the synthesis of a protein is mediated by mRNA.

information carrier that codes for the synthesis of one or more proteins. Proteins can be synthesized using the information in mRNA as a template (Figure 1). Downstream genes can be upregulated or downregulated, turned on or off by messenger proteins.

Many factors determine whether a gene is on or off, such as the time of day, whether or not the cell is actively dividing, its local environment, and chemical signals from other cells. Upregulation and downregulation of genes affects the very wiring of the brain and body, predisposing the body toward the development of disease, or improving health, thinking, and memory (Church, 2013c; Montag-Sallaz et al., 1999; Ramanan et al., 2005; Pfenning, Schwartz, & Barth, 2007; Yehuda et al., 2009; Brocke et al., 2010).

Information transfer between DNA, RNA (both nucleic acids), and protein is multidimensional and occurs in several different ways. There are direct transfers of information between DNA, RNA, and proteins. DNA can be copied to DNA (replication). DNA information can be copied into mRNA (transcription). mRNA then carries a copy of DNA to other DNA, binding to it and triggering its expression (gene expression). In general, gene expression is regulated through changes in the number and type of interactions between proteins that collectively influence the transcription of DNA and the translation of RNA (Strachan & Read, 1999).

Telomeres are the molecular tails of DNA strands. Each time DNA replicates, a pair of telomerase molecules is lost (Sprung, Sabatier, & Murnane, 1996; Ning et al., 2003). Telomere tails shorten at a stable rate of about 1% per year, and are regarded as the most accurate biological marker of aging (Church, 2013c). Positive changes in lifestyle, such as meditation and a healthy diet, can ameliorate the oxidative effects of stress and preserve telomere length, mitigating the aging process. Telomere length has been correlated with age-related health decline as well as how health is negatively or positively affected by the environment, stressful experiences, and meditation (Kotrschal, Ilmonen, & Penn, 2007; Okereke et al., 2012; Ladwig et al., 2013; Epel, 2009; Epel, Daubenmier, Moskowitz, Folkman, & Blackburn, 2009; Jacobs et al., 2011).

Emotional Freedom Techniques (EFT)

Emotional Freedom Techniques (EFT) is an evidence-based psychotherapy self-help technique. It has been validated in over 100 studies, meta-analyses, and review papers accessible via an online bibliography (Research.EFTuniverse.com). EFT uses elements of exposure and cognitive therapies, and combines them with acupressure (i.e., fingertip stimulation of acupuncture points). It is described in a manual that has been available since the inception of the method, leading to its uniform application in research, training and certification (Craig & Fowlie, 1995; Church, 2013b). Studies have demonstrated its efficacy for a wide variety of psychological conditions and physical symptoms (Wells, Polglase, Andrews, Carrington, & Baker, 2003; Brattberg, 2008; Karatzias et al., 2011; Church, De Asis, & Brooks, 2012; Church, Yount, & Brooks, 2012).

Meta-analyses have found “large” treatment effects for anxiety, depression, and PTSD (Clond, 2016; Nelms & Castel, 2016; Sebastian & Nelms, 2016). The treatment time frames described in these reviews were brief, ranging from one session for phobias to between four and 10 sessions for PTSD. The treatment effects of EFT were found to extend over time. Systematic review papers have also described the efficacy of EFT for pain, traumatic brain injury, sports performance, fibromyalgia, and other physical conditions (Church, 2013b; Feinstein, 2012; Feinstein & Church, 2010). The effect sizes for EFT found in meta-anlyses are larger than those typically observed in conventional psychotherapy and psychopharmacology trials (Clond, 2016; Nelms & Castel, 2016; Sebastian & Nelms, 2016). Several dismantling studies have isolated the acupressure component of EFT from the conventional cognitive and exposure protocols that EFT shares with other therapeutic methods (reviewed in Church & Nelms, 2016). They find that the acupuncture point stimulation element of EFT is an active ingredient and not simply an

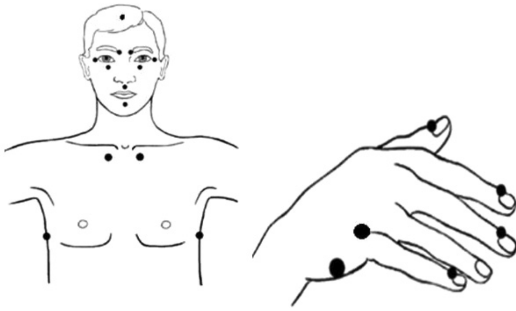


Figure 2. Acupressure points prescribed in *The EFT Manual* (Church, 2013b).

inert placebo. This is confirmed by studies using fMRI and other biological measures to investigate the brain's response to acupuncture; all show regulation of the brain regions and brain-wave frequencies associated with fear (Dhond, Yeh, Park, Kettner, & Napadow, 2008; Bai et al., 2009, 2010; Witzel et al., 2011; Liu et al., 2011). EFT has also been shown to regulate cortisol (Church, Yount, & Brooks, 2012). A study of the epigenetic effects of EFT in veterans with PTSD found regulation of six genes including those in the interleukin family that are linked to the stress response (Church, Yount, Rachlin, Fox, & Nelms, 2015).

Besides its cognitive, exposure, and acupressure tapping components, EFT uses a bilateral brain activation strategy called the 9 Gamut Procedure that is hypothesized to increase communication between the right and left hemispheres of the brain through the corpus callosum. Before and after the application of EFT, clients self-assess their degree of stress on an 11-point Likert scale. They then use EFT's "Basic Recipe" of acupoint stimulation while vividly recalling a traumatic event. This is followed by the 9 Gamut, then by a second application of the Basic Recipe (Figure 2).

The Viability of Saliva Sampling

Previously in gene expression research, blood samples were needed for profiling and analyses. Easily collected fluids such as saliva were dismissed due to several inadequacies. Saliva was considered to have too much extraneous DNA from viruses and bacteria to discriminate human gene expression (Kumar, Hurteau, & Spivack, 2006; Chiappelli, Iribarren, & Prolo, 2006). Before advances in purification and amplification technology, the quantity of mRNA obtainable from saliva was insufficient to measure significant changes (Bartlett & Stirling, 2003). Further, saliva mRNA samples degraded too quickly (Hu et al., 2008).

More recently, it has been established that saliva as a biological sample has the potential, as an easily collected body fluid, for human gene expression and experience research (Zubakov, Hanekamp, Kokshoorn, van Ijcken, & Kayser, 2008; Zubakov, Boersma, Choi, van Kuijk, & Wiemer, 2010). Technology now exists that allows for saliva collection from participants before and after psychotherapeutic intervention by way of in-vial mRNA purification. Storage, analysis, and comparison can be accomplished at room temperature rather than requiring frozen samples. The study used the Oragene saliva self-collection kit (OrageneRNA for Expression Analysis Self-Collection Kit, dnaGenotek, Ontario, Canada). The Oragene device consists of a proprietary fluid matrix in which samples are stored. This device is most commonly used in the medical and public health sectors for downstream isolation of genomic DNA. The manufacturer's directions are easy to follow; the stabilizing liquid is inside the lid of the vial. For the saliva sample, the participant spits into the tube, the lid is tightened, which releases the stabilizing liquid, and then gentle shaking mixes the stabilizer with the saliva.

The Oragene kit is to date the only all-in-one system for the collection, stabilization, and transportation of high-quality mRNA from saliva (Figure 3). This product literature claims that it yields "high quality total RNA." It is advertised as a noninvasive and easy-to-use self-collection tube that remains stable for months at room temperature; therefore, no sample freezing is necessary.

With recent advances in the field of molecular biology, it is possible to scan the entire genome for gene expression using a more cost effective high-throughput, multiplexed bead-based technology (Yang, Tran, & Wang, 2001; Krutzik &

Figure 3. Saliva sample collection vial.

Nolan, 2003; Elshal & McCoy, 2006; Jacobson, Oliver, Weiss, & Kettman, 2006; Bruse, Moreau, Azaro, Zimmerman, & Brzustowicz, 2008; Leng, McElhaney, Walston, Xie, Fedarko, & Kuchel, 2008). Prior to these advances, it was necessary to look at a very narrow selection of genes, sometimes only one at a time using RT-PCR (Real-Time polymerase chain reaction) technology, which is very costly.

The current study sought to elucidate the effect of psychotherapy on gene expression by measuring expression before and after a single session of EFT. The recent availability of noninvasive saliva tests offers the possibility of elucidating how psychotherapy works as an epigenetic influence on gene expression. The aim of this study was to observe the genetic response of healthy individuals to a psychotherapeutic intervention using saliva sample collection. A second objective was to investigate the feasibility of using saliva rather than blood as a biofluid suitable for conducting genetic research in a clinical setting, and delineate the parameters of a protocol to ensure successful data collection.

Method

Participants and Blinding

The study was approved by the Institutional Review Board (IRB) of Akamai University. Potential participants were English-speaking adults aged 18 to 65. Prior to enrollment, they were screened to verify nonclinical mental health status, and excluded if they scored above 20 on the Brief Symptom Inventory 18 (BSI 18; Derogatis, 2001), adapted for the purpose of this study to include "lifetime" instead of just the last 7 days. Of 24 potential participants screened, 10 were excluded on this basis. Of the remaining 14, five were selected based on their availability for the following two weekends. All provided informed consent.

All study data were de-identified and coded to protect the participants' identities and facilitate impartial analysis of the samples. One participant was disqualified due to admission of a psychiatric diagnosis leaving a final $N = 4$. Sample vials were labeled with five-digit identifying codes determined by a random number generator (http://stattrek.com/statistics/random-number-generator.aspx), thus effectively blinding the molecular biology analysis. The key was not provided to the molecular biology team until after the samples were processed for mRNA extraction, gene detection, and gene expression.

Preliminary Proof of Methods Validation Test

No prior study collecting sufficient quantities of mRNA from samples stored in a preservation matrix at room temperature has been published in the literature. It was necessary to determine whether the mRNA level for a gene in saliva samples was comparable to the level in blood samples in order to demonstrate the feasibility of the planned study. Accordingly, a preliminary validation of the Oragene device was performed. This preliminary investigation tested logistics and procedure feasibility before the launch of the planned study.

For this validation, samples of blood and saliva were collected before, after, 2 hours after, 4 hours after, and 6 hours after a novel experiential psychotherapeutic intervention and stored at room temperature for 2 weeks. mRNA was then extracted from the samples according to manufacturer recommendations. mRNA expression from blood and saliva was quantified for the interleukin-8 (IL8) gene, a pro-inflammatory gene (Shahzad et al., 2010), from the samples using Real-Time PCR (RT-PCR). IL8 was one of the genes found to be significantly regulated by EFT in the prior gene expression study using blood samples analyzed with PCR (Church, Yount, Rachlin, Fox, & Nelms, 2016).

The preliminary validation test found that both saliva and blood were sensitive biofluids, exhibiting significant upregulation, relative to a twofold change threshold, of the IL8 gene from baseline (Figure 4). The Cycle threshold (Ct value) is the number of cycles of PCR amplification at which the signal of the target gene exceeds background noise (Shiao, 2003). mRNA encoding the IL8 gene extracted from saliva was detected at a Ct of 22.

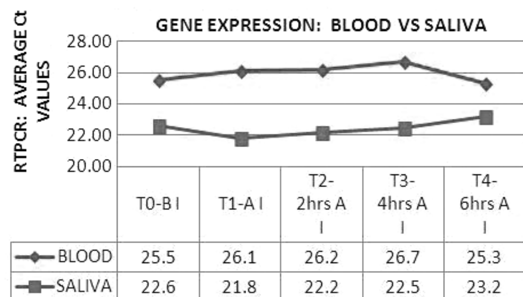


Figure 4. RT-PCR Ct values for IL8 gene expression of blood versus saliva. Time 0, BI = before intervention; Time 1, AI = after intervention; Time 2, AI = 2 hours after intervention; Time 3, AI = 4 hours after intervention; Time 4, AI = 6 hours after intervention.

IL8 mRNA from blood was detected at a Ct of 24. Lower Ct values indicate greater quantities of mRNA. It is expected for biofluids to have different expression patterns for a specific gene as a result of their differing constituents. The similarity of the results obtained from the two biofluids indicated the feasibility of using saliva for the planned study, and demonstrated the logistical feasibility of measuring differential gene expression before and after EFT.

Sample Collection and Analysis

After determining that saliva was a viable biofluid, the main study proceeded. Saliva samples were first collected under control conditions, and then, a week later, from the same participants before and after EFT. The control was 50 minutes of non-therapeutic conversation moderated by a non-therapist research assistant. The experimental condition was 50 minutes of EFT.

Study participants provided 1 ml of saliva by expectorating directly into the Oragene collection vial. Saliva samples were collected from all participants in both groups immediately before (T0), immediately after (T1), 4 hours after (T2), and 24 hours after (T3) the 50-minute treatment. A total of 40 samples were collected one week apart for control and experimental conditions. After collection, vials were sealed and stored at room temperature until RNA extraction.

As these are novel methods, it was decided that an initial trial of 12 samples on one chip would be attempted first. This was performed at the University of Texas Southwestern Microarray Core Lab. The first chip was processed 35 (+ or – 7 days) days after collection. By the time these results had been received and it was determined that quality mRNA had been quantified, only 12 of the next 24 samples passed quality controls. Extraction of the second chip occurred about 80 days after sample collection. dnaGenotek was contacted about the degradation of the samples, which their literature confirmed could be "stored at room temperature for months," and then stated that the expiration point of their collection vials should be considered 60 days.

Labeling, Hybridization, and Data Analysis

The Illumina Human HT-12-V4 BeadChip array is made up of randomly positioned silica beads, each containing hundreds of thousands of copies of a specific probe sequence. From each sample, 50 ng of total RNA was labeled with biotin and then hybridized using the Illumina chip. The chips were then scanned by an Illumina HiScan-SQ scanner. The level of hybridization was measured via Cy3-Streptavidin fluorescence. Data were normalized to background then analyzed using Illumina Genomestudio software (San Diego, CA). This platform was chosen for this study as it allowed for the detection of specific gene expression activity across the entire human genome at a fraction of the cost of using the RT-PCR procedure, yet with comparable detection capabilities.

Statistical Analysis

Using the Illumina Genomestudio software, gene expression data were pooled and compared across each condition and time point. Data from individual participants were normalized using quantile normalization. This is a method used to make the distribution, median, and mean of probe intensities the same for every sample. The normalization distribution is chosen by averaging each quantile across samples in order to generate an average signal (AVG-Signal). The data analysis included the p value and other descriptive statistics such as Standard Deviation, Standard Error, and t-test for each gene. The p value indicates the statistical significance that the detected signal on the chip was differentially illuminated when compared to the controls and background noise. Detection p value is a statistical calculation that provides the probability that the signal from a given probe is greater than the average signal from the negative controls. A p value < 0.01 indicates that a specific gene exhibited significant up- or downregulation from controls and background noise.

Genes that were differentially expressed between groups were identified by comparing the expression values of the genes. Differential expression was determined via Student's t-test for the treatment group in question. Differences between conditions (CC = control; EC = experimental) and time point with $p < 0.05$ were considered significant.

Of the 40 samples collected, only 24 samples were suitable for analysis based on extractable mRNA. Obtaining high quality mRNA was problematic due to sample degradation. There was variability in the number of detectable genes found in each sample, displayed in Table 1. This was most

Table 1. *Number of Genes Detected in Each Sample per Condition and Time Point out of 47,000 Genes*

Sample groups	CS	ES
T0	CS5T0: 6410	ES5T0: 416*
	CS4T0: 522*	
	CS3T0: 2781	ES4T0: 618*
	CS2T0: 1585	
	CS1T0: 425*	ES3T0: 3434
T1	CS5T0: 8548	ES5T1: 701*
	CS3T0: 4728	ES2T1: 9064
	CS2T1: 1504	ES4T1: 618*
T2	CS5T2: 10634	ES5T2: 9064
		ES3T2: 516*
T3	CS5T3: 7741	ES5T3: 570*
		ES4T3: 521*
	CS4T3: 709*	ES3T3: 427*
	CS3T3: 1267	ES2T3: 1576

Note: *These samples have a very low number of detectable genes.

likely due to the time lapse between collection and sample processing.

Samples obtained at Time 2, the time point 4 hours after the intervention, did not yield enough usable mRNA to be compared across conditions. The data for this time point have therefore been eliminated from the results due to the poor quality mRNA extraction from most of the samples. Participants were instructed on study procedures and collection times; however, instructions may not have been followed, as this was the only time point that was not observed by the research assistant. It is possible the participants ate, smoked, or chewed gum before collecting that particular sample.

Results

RNA Extraction and Validation

Samples were extracted following the steps listed in the Oragene purification protocol using TRIzol LS reagent (Invitrogen, Carlsbad, CA). Total RNA samples (1 µl) were analyzed for quality and purity by chip electrophoresis using Agilent's 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA) and reagents from the RNA 6000 pico kit. Table 2 shows the number of samples that passed extraction and validation for each time point and condition. The data from Time 2 (T2), 4 hours after the intervention, were eliminated from further analysis due to too few usable samples to compare.

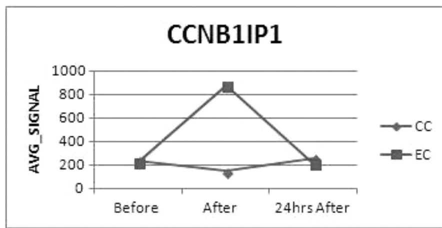
Differential Gene Expression

Differential gene expression was measured using the methods outlined previously. Because of the size and magnitude of the data generated (out of 47,000 genes), only 10 of the 72 differentially expressed genes will be mentioned for the results (Figure 5, Table 3). Downstream investigation is suggested for these 10 differentially expressed genes: CCNB1IP1 is also known as Cylin B1 interacting protein. It is involved with the progression of the cell cycle. CCNB1IP1 showed significant upregulation, 8-fold, immediately after EFT compared to the control condition ($p < 0.02$). Expression of CCNB1IP1 has been linked to tumor suppression (Ma et al., 2013). COPS7A is a subunit of COP9 and is known as constitutive photomorphogenic signalosome subunit 7A. COP9 and its subunits play a role in protecting and repairing damaged DNA due to UV radiation (Füzesi-Levi et al., 2014). COPS7A showed significant upregulation, 5-fold, after EFT compared to control condition ($p < 0.02$). DAB2, Disabled-2, is a FOXP3 target gene for regulating

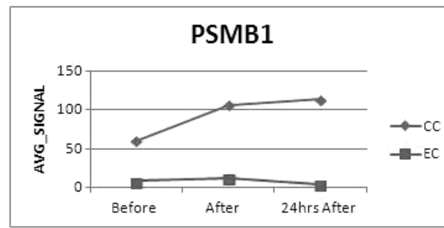
Table 2. *Sample Information*

Sample groups	Samples in CS group		Samples in ES group	
T0	5	CS5T0	3	ES5T0
		CS4T0		
		CS3T0		ES4T0
		CS2T0		
		CS1T0		ES3T0
T1	3	CS5T0	3	ES5T1
		CS3T0		ES2T1
		CS2T1		ES4T1
T2	1	CS5T2	2	ES5T2
				ES3T2
T3	3	CS5T3	4	ES5T3
				ES4T3
		CS4T3		ES3T3
		CS3T3		ES2T3

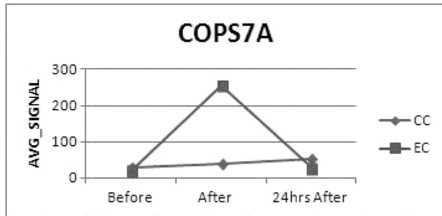
Note: Total 24 out of 36 samples were processed on Illumina Human HT-12 arrays.



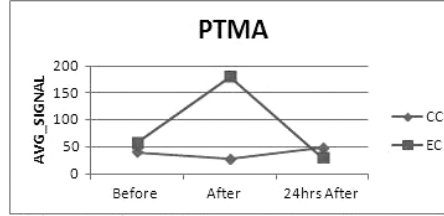
Cyclin B1 interacting protein 1, E3 ubiquitin protein ligase: progression of the cell cycle.



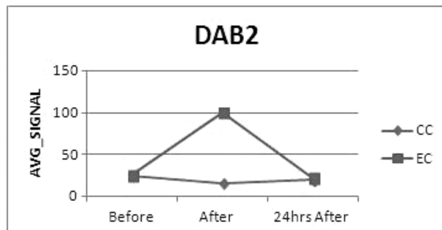
Proteasome, subunit beta type; insulin regulation.



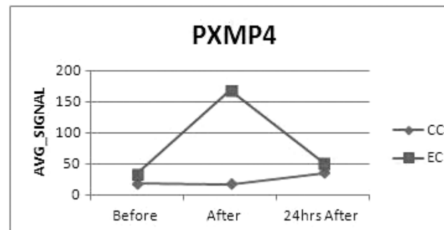
Constitutive Photomorphogenic Signalosome subunit 7A; cellular processes.



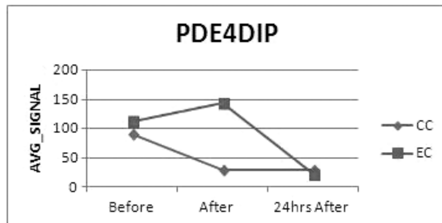
Prothymosin alpha mediates immune function; resistance to opportunistic infections.



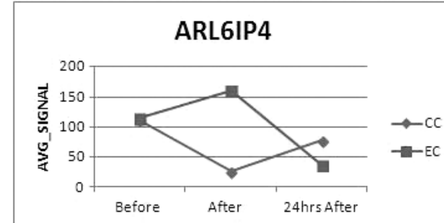
Disabled-2 is a FOXP3 target gene required for regulatory T cell function.



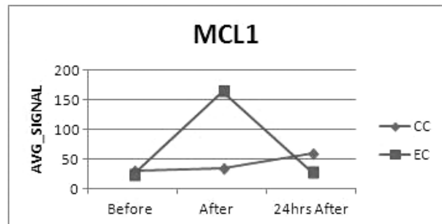
Peroxisomal Intrinsic Membrane Protein; tumor suppression.



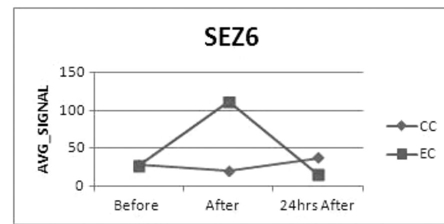
Phosphodiesterase 4D interacting protein; mood, memory & learning.



ADP ribosylation factor like GTPase 6 interacting protein 4; antiviral activity.



Myeloid Cell Leukemia 1: Mediates apoptosis (programmed cell death).



Seizure-related gene 6; enhanced synaptic connectivity.

Figure 5. Differential gene expression of 10 genes of interest and basic function: Average signal (AVG_SIGNAL) of control condition (CC) compared to experimental condition (EC).

T-cell function. DAB2 has tumor suppression and anti-cancer effects. Loss of DAB2 expression in breast cancer can be detrimental to prognosis (Xu, Zhu, & Wu, 2014). Downregulation of DAB2 switches TGF-B (tumor growth factor-B) from a tumor suppressor to a tumor promoter (Hannigan et al., 2010). DAB2 showed significant upregulation, 3-fold, after EFT compared to control condition ($p < 0.02$). PDE4DIP, phosphodiesterase 4D interacting protein has implications for mood, memory, and learning (Kim, Cho, Lee, & Webster, 2012; Shapshak, 2012). PDE4DIP upregulated 2-fold immediately after EFT ($p < 0.02$). MCL1, Myeloid Cell Leukemia 1, mediates apoptosis (programmed cell death). MCL1 is named and known for the role it plays in cancer promotion when it is

Table 3. *Summary of 10 Genes and Translational Functions*

Gene	Discovered function(s)	References
CCNB1IP1	Tumor suppression	Ma et al., 2013
COPS7A	Protects against UV radiation	Groisman et al., 2003; Füzesi-Levi et al., 2014
DAB2	Cancer tumor suppression	Tong et al., 2010; Hannigan et al., 2010; Xu, Zhu, & Wu, 2014
PDE4DIP	Implications for mood, memory, and learning	Kim, Cho, Lee, & Webster, 2012; Shapshak, 2012
MCL1	Neuronal survival after DNA damage. Suppression, cancer prevention	Xingyong et al., 2013; Lestini et al., 2009
PSMB1	Increases type 2 diabetes insulin resistance. Upregulation related to anticancer	Yamauchi et al., 2013; Keutgens et al., 2010
PTMA	Mediates immune function by increasing resistance to opportunistic infections. Antiviral properties when upregulated	Su et al., 2013; Bowick et al., 2010
PXMP4	Cancer tumor suppression	Zhang et al., 2010
ARL6IP4	Resistance and recovery from emotional stress and antiviral activity	Wu et al., 2013; Carhuatanta, Shea, Herman, & Jankord, 2014
SEZ6	Enhances synaptic connectivity in the brain by promoting dendritic arborization (branching) of neurons	Gunnersen et al., 2007

overexpressed (Lestini et al., 2009; Ertel, Nguyen, Roulston, & Shore, 2013). With moderate expression, however, MCL1 has been found to help neuronal survival after DNA damage (Xingyong et al., 2013). MCL1 showed significant upregulation after EFT compared to control condition ($p < 0.004$). PSMB1, Protaesome subunit beta type-1, is a multicatalytic proteinase complex with a highly ordered ring-shaped 20S core structure. PSMB1 is known to play a role in insulin resistance when overexpressed (Yamauchi, Sekiguchi, Shirai, Yamada, & Ishimi, 2013). PSMB1 remained constant throughout the experiment, unlike the control condition, which was variable ($p < 0.002$). PTMA, Prothymosin alpha, mediates immune function by increasing resistance to opportunist infections. PTMA showed significant upregulation, 5-fold, after EFT compared to control condition ($p < 0.04$). PXMP4, Peroxisomal Intrinsic Membrane Protein, has been found to have cancer tumor suppression properties when expressed (Zhang et al., 2010). PXMP4 showed significant upregulation, 5-fold, after EFT compared to control condition ($p < 0.04$). ARL6IP4, ADP ribosylation factor like GTPase 6 interacting protein 4, is known for resistance and recovery from emotional stress and antiviral activity (Wu et al. 2013; Carhuatanta, Shea, Herman, & Jankord, 2014). ARL6IP4 upregulated 2-fold after EFT ($p < 0.002$). SEZ6, seizure related gene 6, has implications for enhancing synaptic connectivity in the brain by promoting dendritic arborization, (branching) of neurons (Gunnersen et al., 2007). SEZ6 showed significant upregulation, 3-fold, after EFT compared to control ($p < 0.05$). A basic PubMed search on 32 of the 72 genes differentially expressed in the immediately after EFT condition compared to control condition yielded some very interesting possibilities for how EFT might affect immunity and inflammation systemically (Table 4). Also immediately after EFT, genes were expressed that are known to code for structural neurogenesis and brain plasticity.

Discussion

This study piloted a novel methodology of using saliva to measure mRNA and gene expression before and after therapy in order to test the feasibility of using gene expression to examine the physiological correlates of effective treatment. A broad, genome-wide analysis was performed to detect differential gene expression from saliva samples collected before and after

Table 4. *Functions of 32 of the 72 Genes Differentially Expressed after EFT*

Symbol	ES-T1.diff pval	Gene function	Symbol	ES-T1.diff pval	Gene function
MAL	0.000140196	T cell/immunity	**SLK**	0.01066234	Oral cavity related
PVRL3	0.000726317	Growth/immunity	**RPL19P9***	0.01131552	Cancer tumor regulation
ARL6IP4*	0.002220663	Antiviral activity	**EFCAB6**	0.01169039	Hormone related
PSMB1*	0.002253853	Insulin regulation	**UCP3**	0.01271318	Energy & metabolism related
EIF2B2	0.002633457	Brain white matter regulator	**PPM1G**	0.01599931	DNA methylation; relates to brain function & behavior
DGKD	0.003499107	Cellular response to external stimuli	**KDM6B**	0.01679955	Encodes an epigenetic regulator promoting transcriptional plasticity
SBDS*	0.003522641	Blood cells development & differentiation	**DAB2***	0.01702478	Cancer tumor suppressor
FLJ45337*	0.004008878	CNS	**PDE4DIP***	0.01735445	Memory & learning
GNAI3	0.004695438	Brain pituitary function	**PACSIN2**	0.01792237	Mediates membrane sculpting
PRB3	0.006684133	Saliva related	**GPX1**	0.01820716	One of the most important antioxidant enzymes in humans
RFX2	0.007274315	Male fertility	**COPS7A***	0.0232078	Prevents UV damage
CNTNAP3*	0.008081845	Cell recognition in CNS	**CCNB1IP1***	0.02381662	Tumor suppressor
MEGF10	0.008235929	Brain synapse shape, implicated in mood changes	**SLC25A24***	0.03513861	Cancer promotion/autism & brain damage
LSM1	0.008412043	Regulation of stress response	**MCL1***	0.04546044	Neuronal survival after DNA damage
CASP1	0.009469124	Inflammation & immune response	**SEZ6***	0.04947686	Enhances brain synapse connectivity
NAPRT1	0.009792715	Nicotinic pathway signaling	**PXMP4***	0.04958648	Tumor suppressor

Note: *Stable AVG signal across all conditions and time points.

Table 5. *72 Genes Differentially Expressed in the Immediately after EFT Condition Compared to Control Condition*

Symbol	ES-T1.diff pval	Symbol	ES-T1.diff pval	Symbol	ES-T1.diff pval
MAL	0.000140196	**PDE4DIP**	0.01735445	**TRIOBP**	0.03726178
PVRL3	0.000726317	**PACSIN2**	0.01792237	**PNKP**	0.03818927
ARL6IP4	0.002220663	**GPX1**	0.01820716	**NDUFA4**	0.03842646
PSMB1	0.002253853	**LOC644908**	0.01976341	**HECTD1**	0.03899735
EIF2B2	0.002633457	**BRD2**	0.01978085	**PTS**	0.04000588
DGKD	0.003499107	**CNBP**	0.02002777	**MPPE1**	0.04160427
SBDS	0.003522641	**EML3**	0.02025446	**LOC100128908**	0.04251099
FLJ45337	0.004008878	**DBN1**	0.02191621	**BCL11A**	0.04295282
GNAI3	0.004695438	**CHMP2A**	0.02248847	**LPXN**	0.04303961
	0.005137144	**COPS7A**	0.0232078	**BRP44L**	0.04319492
PRB3	0.006684133	**CCNB1IP1**	0.02381662	**LOC442727**	0.0434711
RFX2	0.007274315	**HAUS4**	0.0239315	**PAQR8**	0.0436732
CNTNAP3	0.008081845	**LPP**	0.02496151	**FLI1**	0.04385494
MEGF10	0.008235929	**FLJ44290**	0.02684345	**PDE8B**	0.04456585
LSM1	0.008412043	**SLC38A2**	0.02692815	**MCL1**	0.04546044
CASP1	0.009469124	**REPS2**	0.02750115	**RILPL2**	0.04632794
NAPRT1	0.009792715	**CCRL1**	0.02955338	**STAT1**	0.04671226
SLK	0.01066234	**LOC100129553**	0.03140679	**LOC650005**	0.04722419
RPL19P9	0.01131552	**PTOV1**	0.03205068	**LOC649049**	0.0483591
EFCAB6	0.01169039		0.0336481	**WWP2**	0.0488626
UCP3	0.01271318	**DBNDD2**	0.03456545	**SEZ6**	0.04947686
PPM1G	0.01599931	**VPS37C**	0.03480522	**PXMP4**	0.04958648
KDM6B	0.01679955	**SLC25A24**	0.03513861	**DPEP3**	0.04997416
DAB2	0.01702478	**FCN1**	0.03625635	**MAFF**	0.05009715

EFT. A preliminary test using an important regulatory gene showed that noninvasive salivary assays can produce results similar to blood sampling. The time points of immediately before, immediately after, 4 hours after, and 24 hours after the intervention provided data that may be useful in developing a simple pre-post protocol. Immediately after EFT, 72 genes were found to be differentially expressed (Table 5). In 24 hours after EFT, 25 genes were found to be differentially expressed (Table 6).

The data indicate that it is possible to use saliva for gene expression studies using the novel methodology employed in this study. Also, many of the differentially expressed genes in this study are shown to be linked to immunity, pro- or anti-inflammatory, and neuronal processes in the brain and body (Kantor, Alters, Cheal, & Dietz, 2004). And further, based on the results of Dusek et al., (2008) and Bhasin et al., (2013), who used blood as the biofluid, it is apparent that emotional regulation elicits gene expression patterns that correlate with positive health states in a non-clinical population. Whereas meta-analyses demonstrate that EFT is an efficacious treatment for psychological conditions such as anxiety, depression, and PTSD, the results of this study are consistent with earlier studies such as Church, Yount, and Brooks (2012) and Church (2014) and indicate that EFT is an epigenetic intervention, regulating physiological as well as psychological health.

Using saliva as a viable biofluid for gene expression research is becoming more feasible as researchers have overcome purity and specificity problems (Kumar, Hurteau, & Spivack, 2006). In 2008, Zubakov, Hanekamp, Kokshoorn, van Ijcken, and Kayser, published research comparing

Table 6. *25 Genes Differentially Expressed in the 24 Hour after EFT Condition Compared to Control Condition*

Symbol	ES-T3.diff pval
LOC728126	0.000857072
CSRP1	0.002486051
CTDSP1	0.00287435
NDUFB8	0.004585848
LOC401115	0.005995638
ASS1	0.009637041
SDC1	0.01055176
ZDHHC7	0.0108301
C20orf3	0.01124804
LMF2	0.02383739
LOC644511	0.02745767
ADPGK	0.02803549
HLX	0.02855908
CCDC28A	0.0299089
LOC643357	0.03101086
SEC61G	0.03351247
YY1AP1	0.04316361
SFRS4	0.04444867
PTOV1	0.04462961
DCTN2	0.04591753
ATP6V0A1	0.04884839
SDF2L1	0.04910131
LOC730255	0.04998559
CENTD2	0.05004921
LOC644464	0.05011049

blood and saliva (with microarray and RT-PCR validation), successfully demonstrating time-wise sample-specific differential gene expression. In 2013, Pandit, Cooper-White, and Punyadeera sought to demonstrate a high-yield extraction method of mRNA from saliva using a protocol very close to the extraction methods used in the present study. The main difference was that samples were frozen immediately in dry ice. They succeeded in obtaining high-yield and high-quality mRNA from saliva. Furthermore, Xu et al., (2014) were able to identify tissue/sample specific gene expression based on target expression patterns from sample mixtures including saliva. This team's intention was to develop a highly selective and specific multiplex system for mRNA detection from multiple body fluids and tissues. This is useful in forensic applications where multiple fluids may be present or mixed at a crime scene but also supports the aim of this study and future studies in biopsychology.

The limitations of this study include small sample size, the delay in sample processing, sample degradation due to room temperature storage, and possible participant noncompliance at Time 2. The study is further limited by the lack of an active control such as cognitive behavior therapy. Future studies will account for the monitoring of participants at each time point to assure correct sample collection procedures are followed. Studies with a larger *N* will allow for more robust statistical analyses to be performed, such as ANOVA and Rank-Sum testing. All samples should be collected in the presence of study personnel, and frozen immediately following collection to minimize the degradation of mRNA and maximize the quantities of useable genetic material for downstream analysis. Further research should also compare EFT to other efficacious therapies, and include not only biological methods, but also valid and reliable psychological questionnaires. Significant interactions between the symptoms of anxiety and depression on the one hand and the expression of classes of genes such as immunity and inflammation genes on the other hand could be statistically explored.

Using the Oragene device required specific extraction methods that are published and accepted for the device. Oragene has special methods because the RNA must be extracted from the chemical matrix in addition to the saliva. Although saliva is now an acceptable medium for acquiring mRNA, this study had low mRNA detected in some samples as a result of the length of time the samples were stored at room temperature. These were novel methods for this study, but the delay beyond expiration was unexpected. Even with the delay in processing and sample collection device expiration, the fact that quality mRNA, pure and specific, was still able to be extracted from the samples supports the feasibility of conducting research with saliva to validate the effects of psychotherapeutic interventions. Future studies should include freezing the samples on dry ice for shipping to a core facility.

Once larger studies are conducted with solid quality controls and replicable results, the assessment of gene expression as a measure of therapeutic efficacy may become routine. With effective pre and post biological indicators, in

addition to valid and reliable psychometric tools, it could become much easier to measure psychotherapeutic treatment effectiveness. Recent research in epigenetics is exploring in detail how genes and the environment interact specifically related to the measurement of mental and physical health status as it correlates with DNA methylation (Zhang et al., 2010; Jin, Li, & Robertson, 2011; Mehta et al., 2013; Wankerl et al., 2014).

The novel methods employed in the present study also point to the possibility of individualized epigenetic treatment plans. While one client might, for instance, respond best to EFT and yoga, another might respond better to cognitive therapy and mindfulness meditation. Inexpensive and noninvasive salivary assays make personalized medicine possible. Salivary testing facilitates the feasibility of such personalized protocols since they are easily administered, especially compared to the alternative of repeated blood sample collection using a semipermanent cannula for peripheral blood collection (Wankerl et al., 2014). Once the protocols are identified and the costs fall due to increased manufacturing volume, salivary gene tests such as the ones used in this study might become a routine part of psychological assessment.

References

Ackerman, K., Martino, M., Heyman, R., Moyna, N., & Rabin, B. (1998). Stressor-induced alteration of cytokine production in multiple sclerosis patients and controls. *Psychosomatic Medicine, 60*, 484–491.

Ahn, H., & Wampold, B. (2001). Where oh where are the specific ingredients: A meta-analysis of component studies in counseling and psychotherapy. *Journal of Counseling Psychology, 48*(3), 251–257.

Alexander, C. L., Arnkoff, D. B., & Glass, C. R. (2010). Bringing psychotherapy to primary care. *Clinical Psychology: Science and Practice, 17*, 191–214. doi:10.1111/j.1468-2850.2010.01211.x

Anderson, B. L., Farrar, W. B., Golden-Kreutz, D. M., Glaser, R., Emery, C. F., Crespin, T. R., Shapiro, C. L., & Carson, W. E., III. (2004). Psychological, behavioral, and immune changes after a psychological intervention: A clinical trial. *Journal of Clinical Oncology, 22*(17), 3570–3580. doi:10.1200/JOC.2004.06.030

Bai, L., Qin, W., Tian, J., Liu, P., Li, L., Chen, P., … Liu, Y. (2009). Time-varied characteristics of acupuncture effects in fMRI studies. *Human Brain Mapping, 30*(11), 3445–3460.

Bai, L., Tian, J., Zhong, C., Xue, T., You, Y., Liu, Z., … Liu, Y. (2010). Acupuncture modulates temporal neural responses in wide brain networks: Evidence from fMRI study. *Molecular Pain, 6*, 73.

Bartlett, J. M., & Stirling, D. (2003). *PCR protocols* (2nd ed.). Totowa, NJ: Humana Press.

Benson, H., & Klipper, M. (1975). *The relaxation response.* New York, NY: Morrow.

Bhasin, M. K., Dusek, J. A., Chang, B. H., Joseph, M. G., Denninger, J. W., Fricchione, G. L., Benson, H., & Libermann, T. A. (2013). Relaxation response induces temporal transcriptome changes in energy metabolism, insulin secretion and inflammatory pathways. *PLoS One, 8*(5), e62817.

Bowick, G., Soman, K., Wang, H., Aronson, J., Luxon, B., Lomas, L., Gorenstein, D., & Herzog, N. (2010). Proteomic analysis of Pichindé virus infection identifies differential expression of prothymosin-alpha. *Journal of Biomedicine and Biotechnology, 2010*, 956823. doi:10.1155/2010/956823

Boyke, J., Driemeyer, J., Gaser, C., Büchel, C., & May, A. (2008). Training-induced brain structure changes in the elderly. *Journal of Neuroscience, 28*(28), 7031–7035.

Brattberg, G. (2008). Self-administered EFT (Emotional Freedom Techniques) in individuals with fibromyalgia: A randomized trial. *Integrative Medicine: A Clinician's Journal, 7*(4), 30–35.

Brocke, B., Lesch, K. P., Armbruster, D., Moser, D. A., Müller, A., Strobel, A., & Kirschbaum, C. (2010). Stathmin, a gene regulating neural plasticity, affects fear and anxiety processing in humans. *American Journal of Medical Genetics. Part B, Neuropsychiatric Genetics, 153B*(1), 243–251.

Bruse, S., Moreau, M., Azaro, M., Zimmerman, R., & Brzustowicz, L. (2008). Improvements to bead-based oligonucleotide ligation SNP genotyping assays. *BioTechniques, 45*(5), 559–571. doi:10.2144/000112960

Carhuatanta, K., Shea, C., Herman, J., & Jankord, R. (2014). Unique genetic loci identified for emotional behavior in control and chronic stress conditions. *Frontiers in Behavioral Neuroscience, 8*, 341. doi:10.3389/fnbeh.2014.00341

Chiappelli, F., Iribarren, F., & Prolo, P. (2006). Salivary biomarkers in psychobiological medicine. *Bioinformation, 1*(8), 331–334.

Church, D. (2012). *The EFT mini-manual.* Santa Rosa, CA: Energy Psychology Press.

Church, D. (2013a). Clinical EFT as an evidence-based practice for the treatment of psychological and physiological conditions. *Psychology, 4*(8), 645–654. doi:10.4236/psych.2013.48092

Church, D. (2013b). *The EFT manual* (3rd ed.). Santa Rosa, CA: Energy Psychology Press.

Church, D. (2013c). *The genie in your genes* (3rd ed.). Santa Rosa, CA: Energy Psychology Press.

Church, D. (2014). Pain, depression, and anxiety after PTSD symptom remediation in veterans. *Explore: The Journal of Science and Healing, 10*(3), 162–169.

Church, D., De Asis, M., & Brooks, A. (2012). Brief group intervention using emotional freedom techniques for depression in college students: A randomized controlled trial. *Depression Research and Treatment, 2012*, 1–7. doi:10.1155/2012/257172

Church, D., Geronilla, L., & Dinter, I. (2009). Psychological symptom change in veterans after six sessions of Emotional Freedom Techniques (EFT): An observational study. *International Journal of Healing and Caring, 9*(1).

Church, D., & Nelms, J. (2016). Pain, range of motion, and psychological symptoms in a population with frozen shoulder: A randomized controlled dismantling study of Clinical EFT (Emotional Freedom Techniques). *Archives of Scientific Psychology* (in press).

Church, D., Yount, G., & Brooks, A. (2012). The effect of Emotional Freedom Techniques (EFT) on stress biochemistry: A randomized controlled trial. *Journal of Nervous and Mental Disease, 200*(100), 891–896.

Church, D., Yount, G., Rachlin, K., Fox, L., & Nelms, J. (2015). Epigenetic effects of PTSD remediation in veterans using Clinical EFT (Emotional Freedom Techniques): A randomized controlled pilot study. *American Journal of Health Promotion* (in press).

Clond, M. (2016). Emotional Freedom Techniques for anxiety: A systematic review with meta-analysis. *Journal of Nervous and Mental Disease, 204*, 388–395.

Craig, G., & Fowlie, A. (1995). Emotional freedom techniques: The manual. Sea Ranch, CA: Gary Craig.

Derogatis, L. (2001). *BSI 18, Brief Symptom Inventory 18: Administration, scoring, and procedures manual.* Minneapolis, MN: NCS Pearson.

De Roos, C., Greenwald, R., den Hollander-Gijsman, M., Noorthoorn, E., van Buuren, S., & de Jongh, A. (2011). A randomized comparison of CBT and EMDR for disaster-exposed children. *European Journal of Psychotraumatology, 2*, 5694. doi:10.3402/ejpt.v2i0.5694

Dhond, R., Yeh, C., Park, K., Kettner, N., & Napadow, V. (2008). Acupuncture modulate resting state connectivity in default sensorimotor brain networks. *Pain*, *136*(3), 407–418.

Dusek, J. A., Otu, H. H., Wohlhueter A. L., Bhasin, M., Zerbini, L. F., Joseph, M. G., Benson, H., & Libermann, T. A. (2008). Genomic counter-stress changes induced by the relaxation response. *PLoS One, 3*(7), e2576.

Elshal, M., & McCoy, J. (2006). Multiplex bead array assays: Performance evaluation and comparison of sensitivity to ELISA. *Methods, 38*(4), 317–323.

Epel, E. (2009). Telomeres in a life-span perspective: A new "psychobiomarker"? *Current Directions in Psychological Science, 18*(1), 6–10.

Epel, E., Daubenmier, J., Moskowitz, J., Folkman, S., & Blackburn, E. (2009). Can meditation slow rate of cellular aging? Cognitive stress, mindfulness, and telomeres. *Annals of the New York Academy of Sciences, 1172*, 34–53.

Eriksson, P. S., Perfilieva, E., Björk-Eriksson, T., Alborn, A. M., Nordborg, C., Peterson, D. A., & Gage, F. H. (1998). Neurogenesis in the adult human hippocampus. *Nature Medicine, 4*(11), 1313–1317.

Erk, S., Mikschl, A., Stier, S., Ciaramidaro, A., Gapp, V., Weber, B., & Walter, H. (2010). Acute and sustained effects of cognitive emotion regulation in major depression. *Journal of Neuroscience, 30*(47), 15726–15734.

Ertel, F., Nguyen, M., Roulston, A., & Shore, G. C. (2013). Programming cancer cells for high expression levels of Mcl1. *EMBO Reports, 14*(4), 328–336. doi:10.1038/embor.2013.20

Feinstein, D. (2012). Acupoint stimulation in treating psychological disorders: Evidence of efficacy. *Review of General Psychology, 16*(4), 364–380.

Feinstein, D., & Church, D. (2010). Modulating gene expression through psychotherapy: The contribution of non-invasive somatic interventions. *Review of General Psychology, 14*(4), 283–195.

Füzesi-Levi, M., Ben-Nissan, G., Bianchi, E., Zhou, H., Deery, M. J., Lilley, K. S., Levin, Y., & Sharon, M. (2014). Dynamic regulation of the COP9 signalosome in response to DNA damage. *Molecular and Cellular Biology, 34*(6), 1066–1076.

Greenberg, L., & Watson, J. (1998). Experiential therapy of depression: Differential effects of client-centered relationship conditions and process experiential interventions. *Psychotherapy-Research, 8*, 210–224.

Groisman, R., Polanowska, J., Kuraoka, I., Sawada, J., Saijo, M., Drapkin, R., … Nakatani, Y. (2003). The ubiquitin ligase activity in the DDB2 and CSA complexes is differentially regulated by the COP9 signalosome in response to DNA damage. *Cell, 113*(3), 357–367.

Gunnersen, J. M., Kim, M. H., Fuller, S. J., De Silva, M., Britto, J. M., Hammond, V. E., … Tan, S. S. (2007). Sez-6 proteins affect dendritic arborization patterns and excitability of cortical pyramidal neurons. *Neuron, 56*, 621–639. doi:10.1016/neuron.207.09.018

Gutiérrez-Aguilar, M., & Baines, C. (2013). Physiological and pathological roles of mitochondrial SLC25 carriers. *Biochemical Journal, 454*(3), 371–386. doi:10.1042/BJ20121753

Hannigan, A., Smith, P., Kalna, G., Lo Nigro, C., Orange, C., O'Brien, D. I., … Inman, G. J. (2010). Epigenetic downregulation of human disabled homolog 2 switches TGF-beta from a tumor suppressor to a tumor promoter. *Journal of Clinical Investigation, 120*(8), 2842–2857. doi:10.1172/JCI36125

Hölzel, B. K., Carmody, J., Vangel, M., Congleton, C., Yerramsetti, S. M., Gard, T., & Lazar, S. W. (2011). Mindfulness practice leads to increases in regional brain gray matter density. *Psychiatry Research, 191*(1), 36–43.

Hu, Z., Zimmermann, B. G., Zhou, H., Wang, J., Henson, B. S., Yu, W., … Wong, D. T. (2008). Exon-level expression profiling: A comprehensive transcriptome analysis of oral fluids. *Clinical Chemistry, 54*(5), 824–832.

Huang, V., Place, R., Portnoy, V., Wang, J., Qi, Z., Jia, Z., … Li, L. C. (2012). Upregulation of Cyclin B1 by miRNA and its implications in cancer. *Nucleic Acids Research, 40*(4), 1695–1707.

Jacobs, T. L., Epel, E. S., Lin, J., Blackburn, E. H., Wolkowitz O. M., Bridwell D. A., … Saron, C. D. (2011). Intensive meditation training, immune cell telomerase activity, and psychological mediators. *Psychoneuroendocrinology, 36*(5), 664–681.

Jacobson, J. W., Oliver, K. G., Weiss, C., & Kettman, J. (2006). Analysis of individual data from bead-based assays ("bead arrays"). *Cytometry. Part A,* 69(5), 384–390.

Jin, B., Li, Y., & Robertson, K. (2011). DNA methylation: Superior or subordinate in the epigenetic hierarchy? *Genes and Cancer, 2*(6), 607–617.

Kandel, E. (1998). A new intellectual framework for psychiatry. *American Journal of Psychiatry, 155*(4), 457–469.

Kandel, E. (2001). The molecular biology of memory storage: A dialogue between genes and synapses. *Science, 294*(5544), 1030–1038.

Kantor, A., Alters, S., Cheal, K., & Dietz, L. (2004). Immune systems biology: immunoprofiling of cells and molecules. *Biotechniques, 36*(3), 520–524.

Karatzias, T., Power, K., Brown, K., McGoldrick, T., Begum, M., Young, J., … Adams, S. (2011). A controlled comparison of the effectiveness and efficiency of two psychological therapies for posttraumatic stress disorder: Eye Movement Desensitization and Reprocessing vs Emotional Freedom Techniques. *Journal of Nervous and Mental Disease, 199*(6), 372–378.

Keutgens, A., Zhang, X., Shostak, K., Robert, I., Olivier, S., Vanderplasschen, A., … Chariot, A. (2010). BCL-3 degradation involves its polyubiquitination through a FBW7-independent

pathway and its binding to the proteasome subunit PSMB1. *Journal of Biological Chemistry, 285*(33), 25831–25840.

Kim, S., Cho, H., Lee, D., & Webster, M. (2012). Association between SNPs and gene expression in multiple regions of the human brain. *Translational Psychiatry, 2*, e113. doi:10.1038/tp.2012.42

Kotrschal, A., Ilmonen, P., & Penn, D. J. (2007). Stress impacts telomere dynamics. *Biology Letters*, *3*(2), 128–130. doi:10.1098/rsbl.2006.0594

Krutzik, P., & Nolan, G. (2003). Intracellular phospho-protein staining techniques for flow cytometry: Monitoring single cell signaling events. *Cytometry. Part A, 55*(2), 61–70.

Kumar, S., Hurteau, G., & Spivack, S. (2006). Validity of messenger RNA expression analyses of human saliva. *Clinical Cancer Research, 12*(17), 5033–5039.

Ladwig, K. H., Brockhaus, A. C., Baumert, J., Lukaschek, K., Emeny, R. T., Kruse, J., … Peters, A. (2013). Posttraumatic stress disorder and not depression is associated with shorter leukocyte telomere length: Findings from 3,000 participants in the population-based KORA F4 study. *PLoS One, 8*(7), e64762. doi:10.1371/journal.pone.0064762.

Leng, S., McElhaney, J., Walston, J., Xie, D., Fedarko, N., & Kuchel, G. (2008). ELISA and multiplex technologies for cytokine measurement in inflammation and aging research. *Journals of Gerontology. Series A, Biological Sciences and Medical Sciences, 63*(8), 879–884.

Lestini, B., Goldsmith, K., Fluchel, M., Liu, X., Chen, N., Goyal, B., Pawel, B., & Hogarty, M. (2009). Mcl1 downregulation sensitizes neuroblastoma to cytotoxic chemotherapy and small molecule Bcl2-family antagonists. *Cancer Biology and Therapy, 8*(16), 1587–1595.

Liu, J., Qin, W., Guo, Q., Sun, J., Yuan, K., Dong, M., … Tian, J. (2011). Divergent neural processes specific to the acute and sustained phases of verum and SHAM acupuncture. *Journal of Magnetic Resonance Imaging, 33*(1), 33–40.

Ma, Z., Lin, M., Li, K., Fu, Y., Liu, X., Yang, K., … Sun, B. (2013). Knocking down SMC1A inhibits growth and leads to G2/M arrest in human glioma cells. *International Journal of Clinical and Experimental Pathology, 6*(5), 862–869.

Mehta, D., Klengel, T., Conneely, K. N., Smith, A. K., Altmann, A., Pace, T. W., … Binder, E. B. (2013). Childhood maltreatment is associated with distinct genomic and epigenetic profiles in posttraumatic stress disorder. *Proceedings of the National Academy of Sciences U S A, 110*(20), 8302–8307.

Montag-Sallaz, M., Welzl, H., Kuhl, D., Montag, D., & Schachner M. (1999). Novelty-induced increased expression of immediate-early genes c-fos and arg 3.1 in the mouse brain. *Journal of Neurobiology, 38*(2), 234–246.

Nelms, J., & Castel, D. (2016). A systematic review and meta-analysis of randomized and non-randomized trials of Emotional Freedom Techniques (EFT) for the treatment of depression. *Explore: The Journal of Science and Healing* (in press).

Neville, H. J., & Bavelier, D. (2000). Specificity and plasticity in neurocognitive development in humans. *New Cognitive Neurosciences, 2*, 83–98.

Ning, Y., Xu, J., Li, Y., Chavez, L., Riethman, H., Lansdorp, P., & Weng, N. (2003). Telomere length and the expression of natual telomeric genes in human fibroblasts. *Human Molecular Genetics, 12*(11), 1329–1336.

Okereke, O. I., Prescott, J., Wong, J. Y., Han, J., Rexrode, K. M., & De Vivo, I. (2012). High phobic anxiety is related to lower leukocyte telomere length in women. *PLoS One, 7*(7), e40516. doi:10.1371/journal.pone.0040516

Pandit, P., Cooper-White, J., & Punyadeera, C. (2013). High-yield RNA-extraction method for saliva. *Clinical Chemistry, 59*(7), 1118–1122. doi:10.1373/clinchem.2012.197863

Park, N., Li, Y., Yu, T., Brinkman, B., & Wong, D. (2006). Characterization of RNA in saliva. *Clinical Chemistry, 52*(6), 988–994.

Park, N. J., Yu, T., Nabili, V., Brinkman, B. M., Henry, S., Wang, J., & Wong, D. T. (2006). RNAprotect saliva: An optimal room-temperature stabilization reagent for the salivary transcriptome. *Clinical Chemistry, 52*(12), 2303–2304.

Petrella, J., Mattay, V., & Doraiswamy, P. (2008). Imaging genetics of brain longevity and mental wellness: The next frontier? *Radiology*, *246*(1), 20–32.

Pfenning, A., Schwartz R., & Barth, A. (2007). A comparative genomics approach to identifying the plasticity transcriptome. *BMC Neuroscience, 8*, 20.

Ramanan, N., Shen, Y., Sarsfield, S., Lemberger, T., Schutz, G. Linden, D., & Ginty, D. (2005). SRF mediates activity-induced gene expression and synaptic plasticity but not neuronal viability. *Nature Neuroscience, 6*(6), 759–767.

Rossi, E. (2002). *The psychobiology of gene expression: Neuroscience and neurogenesis in hypnosis and the healing arts*. New York, NY: W. W. Norton.

Rossi, E., Rossi, K., Yount, G., Cozzolino, M., & Iannotti, S. (2006). The bioinformatics of integrative medical insights: Proposals for an international psychosocial and cultural bioinformatics project. *Integrative Medicine Insights, 2*, 1–19.

Rutishauser, U., Mamelak, A., & Schuman, E. (2006). Single-trial learning of novel stimuli by individual neurons of the human hippocampus-amygdala complex. *Neuron, 49*, 805–813. doi:10.1016/j.neuron.2006.02.015

Sebastian, B., & Nelms, J. (2016). Emotional Freedom Techniques (EFT) for posttraumatic stress disorder: A systematic review and meta-analysis. *Explore: The Journal of Science and Healing* (in press).

Shahzad, M. M., Arevalo, J. M., Armaiz-Pena, G. N., Lu, C., Stone, R. L., Moreno-Smith, M., … Sood, A. K. (2010). Stress effects on FosB- and interleukin-8 (IL8)-driven ovarian cancer growth and metastasis. *Journal of Biological Chemistry, 285*(46), 35462–s35470.

Shapshak, P. (2012). Molecule of the month, PDE4DIP. *Bioinformation, 8*(16), 740–741.

Shiao, Y. (2003). A new reverse transcription-polymerase chain reaction method for accurate quantification. *BMC Biotechnology, 3*, 22.

Siegel, D. (1995). Memory, trauma and psychotherapy: A cognitive science view. *Journal of Psychotherapy Practice and Research, 4*(2), 93–122.

Siegel, D. (2012). *The developing mind: How relationships and the brain interact to shape who we are* (2nd ed.). New York, NY: Guilford. Sprung, C. N., Sabatier, L., & Murnane, J. P. (1996). Effect of telomere length on telomeric gene expression. *Nucleic Acids Research, 24*(21), 4336–4340.

Strachan, T., & Read, A. (1999). *Human molecular genetics* (2nd ed.). New York, NY: Wiley-Liss.

Su, B., Tseng, Y., Shieh, G., Chen, Y., Shiang, Y., Wu, P., … Wu, C. L. (2013). Prothymosin α overexpression contributes to the development of pulmonary emphysema. *Nature Communications, 4*, 1906. doi:10.1038/ncomms2906

Tong, J. H., Ng, D. C., Chau, S. L., So, K. K., Leung, P. P., Lee, T. L., … To, K. F. (2010). Putative tumour-suppressor gene DAB2 is frequently down regulated by promoter hypermethylation in nasopharyngeal carcinoma. *BMC Cancer, 10*, 253. doi:10.1186/1471-2407-10-253

Waite, W., & Holder, M. (2003). Assessment of the Emotional Freedom Technique: An alternative treatment for fear. *Scientific Review of Mental Health Practice, 2*(1), 20–26.

Wampold, B. E., Mondin, G. W., Moody, M., Stich, F., Benson, K., & Ahn, H. (1997). A meta-analysis of outcome studies comparing bonafide psychotherapies: Empirically, "all must have prizes." *Psychological Bulletin, 122*, 203–215.

Wankerl, M., Miller, R., Kirschbaum, C., Hennig, J., Stalder, T., & Alexander, N. (2014). Effects of genetic and early environmental risk factors for depression on serotonin transporter expression and methylation profiles. *Translational Psychiatry, 4*(e402), 1–9. doi:10.1038/tp.2014.37

Wells, S., Polglase, K., Andrews, H., Carrington, P., & Baker, H. (2003). Evaluation of a meridian-based intervention, Emotional Freedom Techniques (EFT), for reducing specific phobias of small animals. *Journal of Clinical Psychology, 59*(9), 943–966.

Witzel, T., Napadow, V., Kettner, N. W., Vangel, M. G., Hämäläinen, M. S., & Dhond, R. P. (2011). Differences in cortical response to acupressure and electroacupuncture stimuli. *BMC Neuroscience, 12*, 73.

Wu, L., Zhang, X., Che, Y., Zhang, Y., Tang, S., Liao, Y., … Li, Q. (2013). A cellular response protein induced during HSV-1 infection inhibits viral replication by interacting with ATF5. *Science China. Life Sciences, 56*(12), 1124–1133. doi:10.1007/s11427-013-4569-y

Xiang, L., Szebeni, K., Szebeni, A., Klimek,V., Stockmeier, C., Karolewicz, B., Kalbfleisch, J., & Ordway, G. (2008). Dopamine receptor gene expression in human amygdaloid nuclei: Elevated D4 receptor mRNAs in major depression. *Brain Research, 1207*, 214–224.

Xingyong, C., Xicui, S., Huanxing S., Jingsong, O., Yi, H., Xu, Z., Ruxun, H., & Zhong, P. (2013). Upregulation of myeloid cell leukemia-1 potentially modulates beclin-1-dependent autophagy in ischemic stroke in rats. *BMC Neuroscience, 14*, 56. doi:10.1186/1471-2202-14-56

Xu, S., Zhu, J., & Wu, Z. (2014). Loss of Dab2 expression in breast cancer cells impairs their ability to deplete TGF-β and induce Tregs development via TGF-β. *PLoS One, 9*(3), e91709. doi:10.1371/journal.pone.0091709

Xu, Y., Xie, J., Cao, Y., Zhou, H., Ping, Y., Chen, L., … Zhao, Z. (2014). Development of highly sensitive and specific mRNA multiplex system (XCYR1) for forensic human body fluids and tissues identification. *PLoS One, 9*(7), e100123. doi:10.1371/journal.pone.0100123

Yamauchi, J., Sekiguchi, M., Shirai, T., Yamada, M., & Ishimi, Y. (2013). Role of nuclear localization of PSMB1 in transcriptional activation. *Bioscience, Biotechnology, and Biochemistry, 77*(8), 1785–1787.

Yang, L., Tran, D. K., & Wang, X. (2001). BADGE, Beads array for the detection of gene expression, a high-throughput diagnostic bioassay. *Genome Research, 11*(11), 1888–1898.

Yehuda, R., Cai, G., Golier, J., Sarapas, C., Galea, S., Ising, M., … Buxbaum, J. (2009). Gene expression patterns associated with posttraumatic stress disorder following exposure to the world trade center attacks. *Biological Psychiatry, 66*(1), 708–711.

Yount, G. (2013). Energy healing at the frontier of genomics. *Energy Psychology: Theory, Research, and Treatment, 5*(2), 35–40.

Zhang, X., Wu, M., Xiao, H., Lee, M. T., Levin L, Leung, Y. K., & Ho, S. M. (2010). Methylation of a single intronic CpG mediates expression silencing of the PMP24 gene in prostate cancer. *Prostate, 70*(7), 765–776. doi:10.1002/pros.21109

Zubakov, D., Boersma, A., Choi, Y., van Kuijk, P., & Wiemer, E. (2010). MicroRNA markers for forensic body fluid identification obtained from microarray screening and quantitative RT-PCR confirmation. *International Journal of Legal Medicine, 124,* 217–226. doi:10.1007/s00414-009-0402-3

Zubakov, D., Hanekamp, E., Kokshoorn, M., van Ijcken, W., & Kayser, M. (2008). Stable RNA markers for identification of blood and saliva stains revealed from whole genome expression analysis of time-wise degraded sample. *International Journal of Legal Medicine, 122*(2), 135–142.