Intro

At the recent 2013 Evolution of Psychotherapy Congress that took place in California, USA The International Journal ofPsychosocial Genomics: Consciousness and Health Researchwas introduced to more than 8,000 professionals in counselling, psychology, psychotherapy, meditation, mental health, genomics, rehabilitation and translational medicine by our Founding Editors, Mauro Cozzolino, Ernest Rossi, Giovanna Celia and Kathryn Rossi. Ourfounding editors described the scientific and social mission of this new journal with illustrations of the transformations of human consciousness over the past 200 and 100 years. Taken together these illustrations of the deep philosophical and psychobiological transformations of consciousness tell a fascinating story that highlights our motivation for creating The International Journal ofPsychosocial Genomics: Consciousness and Health Research. Our first editorial describes these two illustrations as examples of a new discipline of the digital humanities – the use of computer algorithms to search for meaning in large databases of text and media. This new digital discipline is used to explore 200 years of the cultural history of the transformations of consciousness inover five million digitized books from more than 40 university librariesaround the world. They compare the frequencies of English words like 'religion, spirit, faith and hope,' which have been gradually losing their digital linguistic frequency over the past 200 years while words like 'science, teaching, sex, brain and consciousness have been gaining. What can this mean?

The second illustration provides a hint. It shows that over the past 100 years the words 'DNA' and 'cognitive' shot upward exponentially together while words like 'medicine, meditation, psychotherapy, placebo and hypnosis followed a simple linear path at the bottom. We take this to mean that our DNA and conscious cognitions are now interacting together to create a new mind and global culture. Our journalpresumes to tell this new story of our evolving human nature.

THE INTERNATIONAL JOURNAL OF PSYCHOSOCIAL GENOMICS CONSCIOUSNESS & HEALTH RESEARCH: CELEBRATING THE PSYCHOSOCIAL GENOMIC EVOLUTION OF PSYCHOTHERAPY

A Group Editorial for Our Inaugural Issue
Mauro Cozzolino, Ernest Rossi, Giovanna Celia & Kathryn Rossi

At the recent 2013 Evolution of Psychotherapy Congress that took place in California, USA The International Journal of Psychosocial Genomics, Consciousness & Health Research was introduced to more than 8,000 professionals in counselling, psychology, psychotherapy, meditation, mental health, genomics, rehabilitation and translational medicine by our Founding Editors, Mauro Cozzolino pictured on the left with Ernest Rossi, Giovanna Celia, & Kathryn Rossi.

Who Are We?

These four contributors to the 2013 Evolution of Psychotherapy Congress described the scientific and social mission of the new International Journal of Psychosocial Genomics, Consciousness & Health Research with the following illustrations of the cultural and healing transformations of human consciousness over the past 200 and 100 years. Taken together these two illustrations of the deep philosophical and psychobiological transformations of consciousness tell a fascinating story that highlights our motivation for creating International Journal of Psychosocial Genomics, Consciousness & Health Research at this seminal moment in world awareness of a newly emerging Psychosocial Genomic & Consciousness Research vision for the integrating of the Arts, Culture, Consciousness, Health and Science.

These two transformational graphs of cultural and scientific consciousness illustrate a new discipline of the digital humanities – the use of computer algorithms to search for meaning in large databases of text and media. We used this new digital discipline to explore of 200 years of the cultural history of the transformations of consciousness in over five million digitized books from more than 40 university libraries around the world. We graphically compared the frequencies of English words like 'religion, spirit, faith and hope,' which apparently have been gradually losing their digital linguistic frequency over the past 200 years while words like 'science, teaching, sex, brain and consciousness' have been gaining. What can this mean?

Our impression is that current consciousness on the lower right hand side is an emerging integration of traditional spiritual values with modern education and science. This "free-for-all quest" for the meaning and understanding of life on all levels from mind to genes, molecules and math appears to be facilitating new transformations for people creating their own personal consciousness as well as contributing to the growth of world awareness of how we can support each other in a single global village of health and well being.
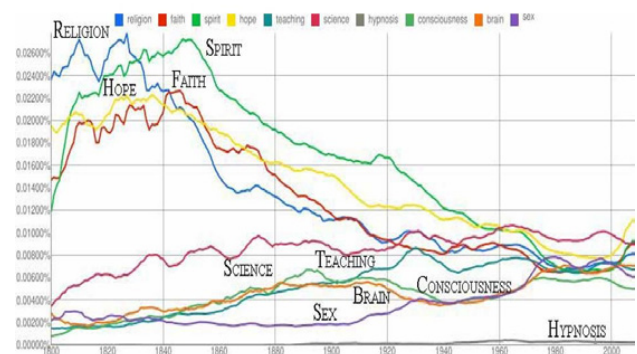
A 200 year history of human efforts to facilitate the cultural and healing transformations of consciousness.

A more current graph of the psycholinguistic transformations of consciousness integrating the 'cognitive' and 'DNA' over 100 years illustrates the emerging synthesis that The International Journal of Psychosocial Genomics, Consciousness & Health Research seeks to facilitate. We invite the entire social and psychobiological community working on all levels from traditional studies of mind, meditation and culture to modern molecular-genomic research on Personal Consciousness, Meaning, Psychology, Education and Translational Medicine to join us so we may go forward together assessing how cognition and genes interact in the new sciences of epigenetics and psychosocial genomics.
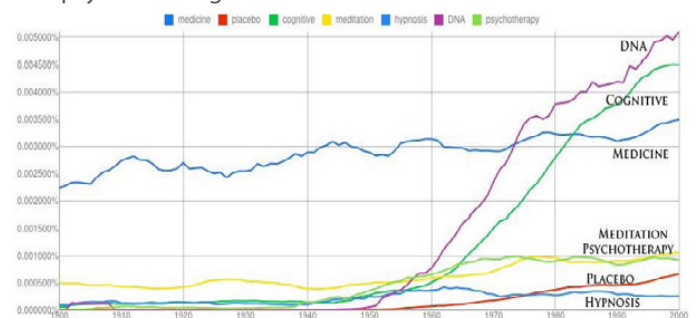
A 100 year Transformation of Consciousness

What Do We Seek to Publish?

While at the present time there is a plethora of open access international scientific journals being introduced and published on the internet at a rapid rate, they are primarily specialized in rather narrow academic disciplines ranging from the artistic, cultural, biological, genomic, molecular, psychological and scientific perspectives. They usually present themselves as holistic and integrative of 'All the Arts and Sciences.' A careful examination of their contents over time, however, usually reveals they are either strongly biased toward a Top-Down Cultural/Literary, Narrative and 'Humanistic' perspective or a Bottoms-Up Molecular/Genomic, Math and 'Scientific' perspective.

All the papers published in this, our inaugural first issue, however, were selected because they brilliantly illustrate what we believe is a 'Truly integrative Top-Down and Bottoms-Up Perspective.' This is well documented by the papers that have some variation of The Four-Stage Psychosocial Genomic Perspective summarized here.

What we very much need are knowledgeable professionals who can contribute basic and introductory papers about the most recent software, methodological and replication techniques for comparing and doing meta-analyses of the vastly expanding scientific literature that attempts to explore the associations between consciousness, cognition and the molecular-genomic underpinning of human experience of art, beauty and truth in general.

Our Editorial Board needs some hard-nosed statistical/math experts in Bayesian Subjective Expectancy as well as Traditional Frequency Statistics for assessing microarrays in the context of new consciousness research about their meaning for educating and enhancing human experience as well as translational medicine.

Of course, not every paper we publish can contribute equally to such a fully integrative Top-Down and Bottoms-Up Perspective. Most humanistic scholars and scientific researchers are, of necessity, specialized in one direction or the other. None-the-less we seek to facilitate the joint ideals of accessibility and meaning for all people – all readers and students of The International Journal of Psychosocial Genomics, Consciousness & Health Research.

We certainly will accept scholarly and scientific submissions that are specialized in their focus on integrating only a few aspects of The Four-Stage Psychosocial Genomic Perspective summarized above. We would hope, however, that the Abstract, Introduction and Conclusion Sections of every paper we publish would explicitly and transparently orient the reader to the broader implications and significance of their work for the welfare of the general public as well as all scientists in general.

This is the idealistic signature that distinguishes the scientific and social mission of The International Journal of Psychosocial Genomics, Consciousness & Health Research.

Please contact us with your impressions of the spirit of our new journal. Email your comments and original contributions directly to our Founding Editors Mauro Cozzolino at mcozzoli@unisa.it and Ernest Rossi at Ernest@ErnestRossi.Com.

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Founding Editors

A BIOINFORMATIC ANALYSIS OF THE MOLECULAR-GENOMIC SIGNATURE OF THERAPEUTIC HYPNOSIS

Mauro Cozzolino, Salvatore Iannotti, Stefano Castiglione, Angela Cicatelli, Kathryn Rossi, Ernest Rossi

ABSTRACT

BACKGROUND:
Although therapeutic hypnosis has been used to treat a range of psychiatric
problems, its biological mechanisms remain poorly understood. We utilized gene microarrays
and bioinformatics software to explore the molecular-genomic basis of therapeutic hypnosis. We
hypothesized that therapeutic hypnosis would be associated with the expression of immediate-
early genes associated with novel stimuli, growth, and psychoneuroimmunology.

METHODS:
Three human subjects experienced a single session of therapeutic hypnosis conducted viaan established protocol (Rossi, 2004a). Gene microarrays assessed the expression of 15,508 genes from each subject's leukocyte RNA immediately prior to, 1 hour after, and 24 hours after the session. RESULTS: Utilizing Gene Set Enrichment Analysis (GSEA), we identified three immediate-early gene sets significantly enriched after 1 hour (p<0.010) and 24 hours (p<0.014). Exploratory analysis documented that our therapeutic hypnosis-induced gene expression profile was:
(1) concordant with a molecular-genomic signature of stem cells after
both 1 hour and 24 hours (p<0.001),
(2) discordant with molecular-genomic signatures induced in states of ultraviolet irradiation after both 1 hour and 24 hours (p<0.013), and
(3) discordant with states of chronic inflammation after 1 hour (p<0.013), but not 24 hours (p=0.148).

CONCLUSIONS:
This study is a proof-of-principle exploration of the molecular-genomic underpinnings of therapeutic hypnosis. Gene expression patterns characteristic of immediate-early gene and enhanced stem cell activity, with reduced cellular stress and inflammation, were found in response to a single session of therapeutic hypnosis. Replication of these top-down, mind-to-molecule mechanisms is essential to developing our understanding of the epigenetic psychosocial genomics of therapeutic hypnosis.

INTRODUCTION

Historically, therapeutic hypnosis has been utilized in the amelioration of numerous medical and psychiatric problems, such as anxiety disorders, depression, pain, substance abuse, stress and post-traumatic stress disorders (Rossi, 2002, 2004a; Tinterow, 1970).

The biological mechanisms underlying this wide range of therapeutic applications remain poorly understood, however. Recently it has been hypothesized that these therapeutic effects hypnosis are mediated, at least in part, by changes at the level of gene expression and cellular physiology (Rossi, 2002, 2007). In a recent "Pilot Study of Positive Expectations and Focused Attention via a New Protocol for Optimizing Therapeutic Hypnosis and Psychotherapy Assessed with DNA Microarrays" we documented changes in the expression of 15 genes within one hour, and subsequent change in the expression of 77 genes 24 hours later (Rossi et al., 2008a).

Within the neuroscience literature on learning, brain plasticity, and memory consolidation, there is growing evidence that novel activity and experience during awake states lead to increased expression of activity and experience-dependent gene expression during subsequent REM sleep states (Ribiero et al. 1999, 2002).
In particular, the immediate early gene Zif-268 (early growth response 1) has been implicated in memory, learning, and exposure to novel and salient life experiences (Ribeiro et al. 1999, 2002. 2004, 2008; Rossi, 2002, 2004a,b, 2007, 2009; Rossi et al. 2008a,b, 2009a,b).
Zif-268 has also been implicated in the immune response (McMahon and Monroe, 1996; Cho et al., 2006). Potentially, the psychobiological underpinnings of therapeutic hypnosis could be related to cellular mechanisms involving similar immediate-early gene responses.
This current paper expands our previous report (Rossi et al., 2008a) to assess the hypothesis that therapeutic hypnosis is associated with measurable, meaningful changes in the level of the gene expression.
We utilized Gene Set Enrichment Analysis (GSEA), a publicly available computational method, to determine whether two sets of genes show statistically significant differences between two biological conditions (http://www.broadinstitute.org/gsea/index.jsp).

GSEA can detect subtle changes in individual gene expression over time and experimental conditions, which collectively result in functionally meaningful up-regulation or down-regulation of established cellular pathways and biological processes (Mootha et al., 2003; Submaranian et We examined whether gene sets related to the activity of Zif-268 / EGR1, were significantly altered in the peripheral leukocytes of human subjects following therapeutic hypnosis. We further compared the gene expression profile found in therapeutic hypnosis with GSEA's database of gene sets implicated in cellular processes (growth, differentiation), disease states (cancer, chronic inflammation), and conditions associated with exposure to toxins and irradiation (Submaranian et al., 2005).

METHODS AND MATERIALS

Patient Characteristics

Our cohort of three highly susceptible hypnotic subjects (2 female, 1 male; median age 34) all had advanced academic degrees and were recruited from a university environment by the Mind Body Institute of San Lorenzo Maggiore (BN) which is part of the Iannotti-Rossi Foundation. Specifically, recruitment was done by the second co-author on the basis of a General Psychiatric Evaluation, The Minnesota Multiphasic Personality Inventory (MMPI-2), The Tellegen Absorption Scale (highly correlated with Standard Scales of Hypnotic Susceptibility), and the Spiritual Intelligence Self Report Inventory (high scorers on the SISRI-24 acknowledge heightened experiences related to Critical Existential Thinking, Personal Meaning Production, Transcendental Awareness, and Conscious State Expansion). Our subjects scored within the normal range of personality characteristics with no evident psychopathology on the MMPI-2.
They were all enthusiastic volunteers who responded well to the "The Creative Psychosocial Genomic Healing Experience" with a positive sense of focused attention, expectancy, appreciation, curiosity, and therapeutic well being. Their past medical history and general physical condition were well-known to the physician involved in the study, and none of the subjects had a history of any major medical illness.

Institutional Approval and Informed Consent

All aspects of the study were approved by the Iannotti-Rossi Foundation and the Ernest Lawrence Rossi Foundation. As all subjects had multiple prior experiences with therapeutic hypnosis facilitated by the psychotherapist in this study, each subject was familiar with the potential risks and benefits of the hypnosis process.
In addition, all subjects received detailed explanations about the reasons for the study, the potential risks and complications associated with phlebotomy, and were informed about their right to withdraw from the study or refuse treatment at any time. No adverse events were experienced by any of the patients throughout the course of the study.

Sample Collection and Processing

All phlebotomies were performed by a Registered Nurse working full time as a phlebotomist at a nearby psychiatric hospital.Peripheral blood was obtained from the three subjects immediately prior to, 1 hour after, and 24 hours after a single session of therapeutic hypnosis according to the protocol "The Creative Psychosocial Genomic Healing Experience" formulated by Rossi (2002, 2004a). Additional description of the sample collection and processing can be found in Rossi et al. 2008a. Briefly, total RNA was extracted from leukocytes, quantified, and purified. Approximately 2.5 µg of purified total RNA was delivered to the MicroCRIBI Service (University of Padova, Italy) for microarray analysis. MicroCRIBI Service performed the microarray analysis on 21,329 - 70mer oligonucleotides (Operon version 2.0) designed on Human Unigene clusters. For each sample, 1.0 µg of total RNA was reverse transcribed and labeled with Cy3 and Cy5 fluorophores for two-channel scanning. Fluorophore labeling of "control" (Immediately before hypnosis) versus "treated" (1 hour or 24 hours after hypnosis) samples was counterbalanced, to control for dye bias. The microarrays were scanned with a two channel confocal microarray scanner (ScanArray# Lite, Perkin Elmer,USA) using its dedicated software (ScanArray Express 3.0.0.,Perkin Elmer).

Preparation of microarray data for GSEA

Channel signals for each probe were initially normalized to background by the scanner software. To control for inter-array variability for each patient as well as for each replicate within a single patient, z-score distributions of probe expression levels were calculated for each patient, at each time point and replicate. Within each patient's data, z-scores were then averaged across replicates for each time point, and a difference of z-scores was obtained by subtracting the pre-treatment z-score expression levels (0 hour time point) from the 1 hour or 24 hour post-treatment z-score expression levels.
Finally, for each probe at each time point, these z-score differences were averaged across all patients, thus resulting in a single, mean z-score difference for each probe at the 1 hour and 24 hour time points.
Probe expression levels were rank-ordered from most positive z-score difference to most-negative z-score difference. All subsequent analyses were performed on these two ranked-lists of probe expression: (1) 1 hour versus 0 hour, and (2) 24 hour versus 0 hour.

Gene Set Enrichment Analysis (GSEA)

Using GSEA, we examined our ranked list of genes to see whether transcription factor targets of the activity and experience-dependent gene Zif-268 (early growth response 1 or EGR1), as well as isoforms EGR 2, 3, and 4, were significantly enriched following therapeutic hypnosis. Gene sets were obtained from the Molecular Signatures Database of transcription factor targets: "Gene sets that contain genes that share a transcription factor binding site defined in the TRANSFAC (version 7.4, http://www.gene-regulation.com/) database."

(http://www.broadinstitute.org/gsea/msigdb/index.jsp). After filtering out gene sets containing fewer than 120 genes, 34 gene sets remained for analysis. Prior to running the analysis, we collapsed probe IDs to gene symbols (using the GSEA "median_of_probes" algorithm), for a final list of 15,508 genes in our dataset.

Other parameter settings in GSEA were as follows: Normalization = meandiv; Scoring = weighted; # of gene set permutations = 1000.

We also compared our ranked lists against the GSEA curated gene sets database, which contains gene sets collected from online pathway databases, publications in PubMed, and knowledge of domain experts (Submaranian et al., 2005). All 192 gene sets containing at least 120 genes were used in the analysis, probes were collapsed to gene symbols using the "median_of_probes" algorithm, and parameters included: Normalization = meandiv; Scoring = weighted; # gene set permutations = 1000.

Gene sets were considered significantly enriched with a combined nominal p-value < 0.02 and false-detection rate (FDR) q-value < 0.20. For selected gene sets achieving this significance level, we used the Leading Edge tool in GSEA to identify and compare those subsets of gene set members that were contributing most strongly to the observed enrichments, within individual gene sets as well as between them. Further analysis was done in Microsoft Excel to then determine which of these genes in common were present in the leading edges of enriched sets at both 1 hour and 24 hours. To examine for time-dependent differences in gene enrichment within individual gene sets at 1 hour versus 24 hours, paired, two-tailed t-tests were calculated.

RESULTS

Figure 1 shows the expression profile of 15,508 genes within 1 hour (x-axis) and 24 hours (y-axis) of therapeutic hypnosis. These z-score difference expression values for each gene were obtained by using the "median_of_probes" algorithm in GSEA to collapse 21,329 probe IDs to 15,508 gene symbols. There was a strong correlation between an individual gene's expression at 1 hour and at 24 hours (Pearson's r > 0.80, p < 0.001).
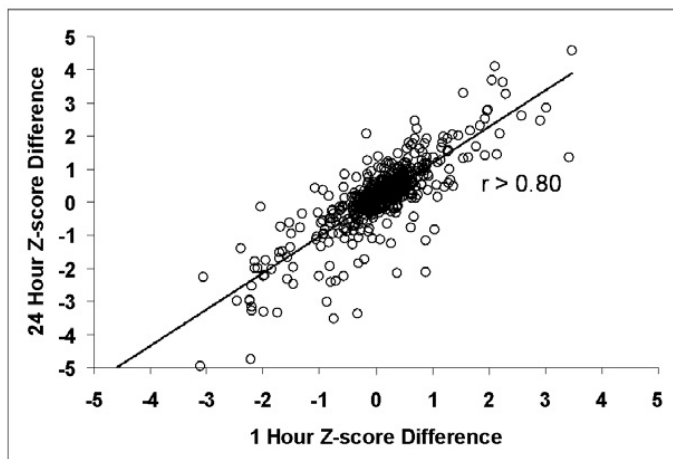
Figure 1. Normalized expression values for the change in the expression of 15,508 genes 1 hour and 24 hours after therapeutic hypnosis. Each data point represents the expression of a single gene, with normalized expression at 1 hour plotted on the x-axis, and normalized expression at 24 hours plotted on the y-axis. Gene expression at 1 hour and 24 hours is strongly correlated (Pearson's r coefficient > 0.80, p < 0.001).

Zif-268 (EGR1) and EGR 2, 3, 4-related gene set enrichment

Within the database of transcription factor target gene sets, three sets of genes with nearby promoter targets of Zif-268 (EGR1) and isoforms (EGR 2, 3, 4) were significantly enriched at 1 hour (all p's < 0.010, all FDR's < 0.063) and 24 hours (all p's < 0.014, all FDR's < 0.095) (see Table 1).

Analysis of curated gene set database

From our exploratory analysis of the GSEA curated gene set database, a set of 290 genes "up-regulated in mouse mature blood cells from adult bone marrow, compared to hematopoietic progenitors" (HSC_MATURE_ADULT, from Ivanova et al., 2002) were significantly enriched after 1 hour (p < 0.001, FDR < 0.151) and 24 hours (p < 0.001, FDR < 0.001). This enrichment was greater at 24 hours than at 1 hour (paired t-test p < 0.0007) (see Table 2 and Figure 2a).
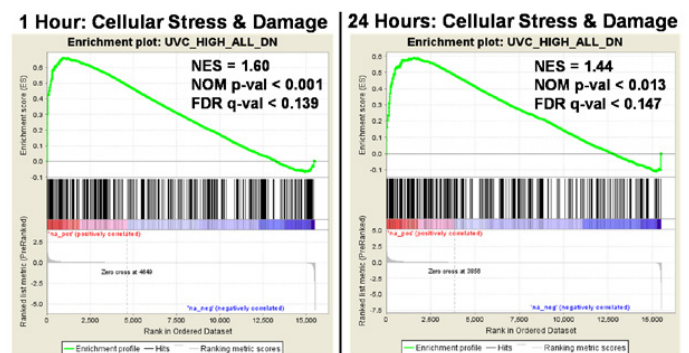
Also from our exploratory analysis, a set of 271 genes down-regulated in human fibroblasts following high dose UVC irradiation (UVC_HIGH_ALL_DN, from Gentile et al., 2003) were positively associated (overall up-regulated) at 1 hour and 24 hours following therapeutic hypnosis. This effect was significant at both 1 hour (p < 0.001, FDR < 0.139) and 24 hours (p < 0.013, FDR < 0.147), and there was no significant difference between the enrichment at the two timepoints (paired t-test p > 0.8) (see Table 2 and Figure 2b).
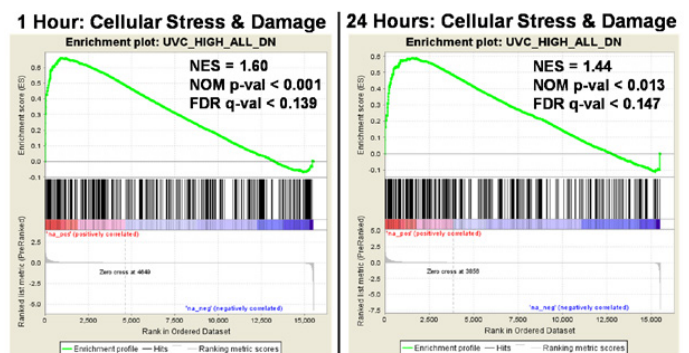
Finally, a set of 139 genes found to be up-regulated in lung tissue of smokers with COPD versus smokers without COPD (NING_COPD_UP, from Ning et al., 2004) were found to be negatively associated with therapeutic hypnosis after 1 hour (p < 0.013, FDR < 0.231) but not 24 hours (p = 0.148, FDR = 0.712). This decrease in enrichment from 1 hour to 24 hours was significant (paired t-test p < 0.013) (see Table 2 and Figure 2c).

Tabel 1

GENE SET	DESCRIPTION: Genes with promoter regions near transcription start sites…	# OF GENES	1 HOUR			24 HOURS		
			NES	NOM p-val	FDR q-val	NES	NOM p-val	FDR q-val
V$EGR_Q6	containing a motif matching annotation for EGR1, EGR2, EGR3	215	1.67	0.004	0.017	1.64	0.001	0.039
V$NGFIC_01	containing a motif matching annotation for EGR4: early growth response 4	196	1.55	0.010	0.045	1.54	0.014	0.066
V$EGR1_01	containing a motif matching annotation for EGR1: early growth response 1	203	1.51	0.008	0.063	1.49	0.006	0.095

Table 1. Results of Gene Set Enrichment Analysis (GSEA) of three gene sets associated with the immediate-early gene Zif-268 (early growth response 1; EGR 1), and related forms (EGR 2,3,4). Gene set names correspond to designations used in the Molecular Signatures Database (http://www.broadinstitute.org/gsea/msigdb/index.jsp). NES = Normalized Enrichment Score = actual ES / mean(ESs against all permutations of the dataset; NOM p-val = Nominal p-value; FDR q-val = False Detection Rate q-value.

Tabel 2

GENE SET	DESCRIPTION	# OF GENES	1 HOUR			24 HOURS		
			NES	NOM p-val	FDR q-val	NES	NOM p-val	FDR q-val
HSC_MATURE_ADULT	Up-regulated in mouse mature blood cells from adult bone marrow	290	1.62	0.001	0.151	1.84	0.001	0.001
UVC_HIGH_ALL_DN	Down-regulated in fibroblasts following high-dose UVC	271	1.60	0.001	0.139	1.44	0.013	0.147
NING_COPD_UP	Up-regulated in lung tissue of smokers with COPD	139	-1.63	0.013	0.231	-1.25	0.148	0.712

Table 2. Results of exploratory analysis of our data with curated gene sets in the Molecular Signatures Database. Gene set names listed correspond to designations used in the Molecular Signatures Database (http://www.broadinstitute.org/gsea/msigdb/index.jsp). NES = Normalized Enrichment Score = actual ES / mean(ESs against all permutations of the dataset; NOM p-val = Nominal p-value; FDR q-val = False Detection Rate q-value.
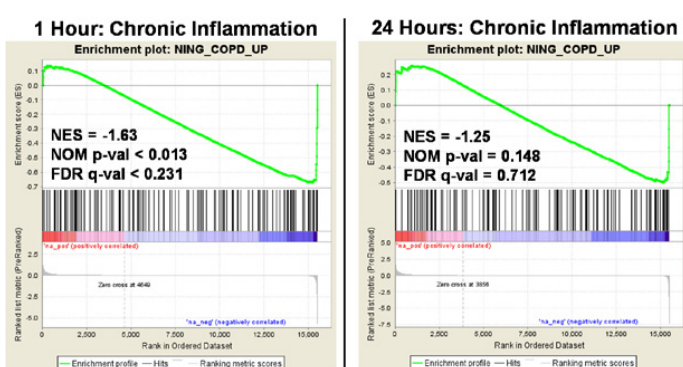
stem cell gene up regulation, and anti-cell stress/DNA damage gene expression patterns(see Figure 3).

These 73 genes were all within the top 10% percent of the ranked lists of genes at both 1 hour and 24 hours.
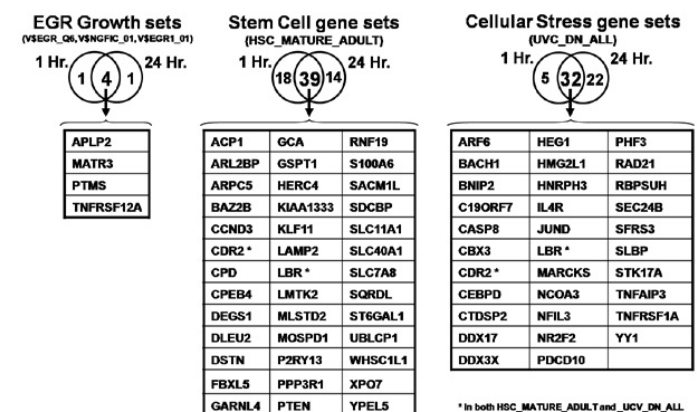
Developing of a Novel Gene Set for Therapeutic Hypnosis

Based upon overlaps at 1 and 24 hours for the positively enriched gene sets (VEGR_Q6, VNGFIC_01, V$EGR1_01, HSC_MATURE_ADULT, UVC_DN_ALL), we created a list of 73 genes that contribute to a collective phenotype of immediate-early gene Zif-268/EGR activity,

DISCUSSION

This pilot study requires replication with more subjects and clinical populations to establish the range, limitations, and utility of exploring the molecular-genomic profiles of experience-dependent gene expression in psychiatry and psychology.

To our knowledge, this is the first proof of principle study to utilize gene microarrays and bioinformatics software to explore the molecular-genomic underpinnings of therapeutic hypnosis. Our data suggests that a single session of therapeutic hypnosis, utilizing The Creative Psychosocial Genomic Healing Experience Protocol (Rossi, 2004a), is associated with gene expression patterns characteristic of immediate-early gene and enhanced stem cell activity, with reduced cellular stress and inflammation. Our analysis suggests that stem cell gene activation continues developing for at least 24 hours following therapeutic hypnosis, whereas the expression of genes related to reduced cellular stress and inflammation may peak within the first few hours and decline thereafter. Further research is now required to characterize these time-dependent effects of therapeutic hypnosis with a variety of between-subjects controls. Interestingly, our findings of alterations in gene expression related to cellular processes of growth and oxidative stress after a single session of therapeutic hypnosis share some similarity with the findings of other researchers who have examined gene expression correlated with the long-term practice of techniques such as Sudarshan Kriya breathing (Sharma et al., 2008) as well as the relaxation response (Dusek et al., 2008). Thus, an additional goal of future research will be to examine the similarities and differences in gene expression profiles of various psychotherapeutic and mind-body health modalities.

Within the limitations of this pilot study, we employed several strategies to increase sampling and reduce experimental error. Each microarray (which contained each probe in duplicate) was run in duplicate or triplicate for each patient, and we calculated the mean expression of each individual probe at the 1 hour and 24 hour time points as relative to the expression of that same probe at 0 hours for the same patient, prior to combining data across patients. By using a within-subjects design, standardizing the data using z-score distributions within each microarray, and alternating the assignment of Cy3 or Cy5 fluorophores to control (0 hr) versus treated (1 hour or 24 hours), we controlled for inter-subject as well as inter-array, inter-sample, and fluorophore labeling/strength variability. Additionally, we collapsed data from probes to genes using the "median of probes" algorithm in GSEA, to avoid counting a single gene more than once, and to additionally serve as a method of repeated sampling for those genes with multiple probes mapped to them. Finally, as we were examining several thousand data points, we chose to examine only those gene sets containing a large number of genes (>120), to minimize Type I error.

It should not escape our notice that the proposed molecular-genomic signature of therapeutic hypnosis may be but one didactic example of an emerging bioinformatic paradigm for the theory, research, and practice of psychiatry, psychology, and psychotherapy. Defining the molecular-genomic signatures for varying states of consciousness, sensation, perception, memory, learning and dreaming, as well as the phenomenology of human experience in general, could well make a contribution to an integrated vision of the arts, humanities, and sciences on all levels from mind to gene.

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A NOVEL MOUTHWASH PROTOCOL FOR NONINVASIVE GENOMIC ANALYSES

Garret Yount and Kenneth Rachlin

ABSTRACT

Peripheral blood cells provide a window into the communication between the mind and body at the genomic level. Progress in this area has been slow, however, primarily due to limitations associated with venipuncture for drawing blood. This study developed a noninvasive, mouthwash-based protocol for collecting neutrophils and harvesting ribonucleic acid (RNA). The RNA Integrity Numbers (RIN) determined for two independent RNA samples obtained for salivary neutrophils collected from the same subject were 9.0 and 8.8, and expression levels for the interleukin 1 beta and interleukin 8 genes were reliably measured by quantitative real-time reverse-transcription polymerase chain reaction analysis. In conclusion, harvesting RNA of sufficient quality for gene expression analyses from salivary neutrophils collected by a mouthwash-based protocol is feasible. Further characterization is needed to determine optimal conditions for investigating differential gene regulation, including characterization of the most reliable reference genes for normalizing data obtained from this subpopulation of blood cells.

INTRODUCTION

Blood cells are an ideal source for exploring how communication takes place between the mind and the body at the level of gene expression, due to their circulation throughout the body and trafficking across the blood-brain barrier [1]. One of the first experiments of this type used an academic stress model to study changes in the immune response associated with psychological stress. Glaser and colleagues collected peripheral blood samples from medical students at the time of exams and at a baseline period one-month prior. Consistent with previous findings demonstrating stress-associated depression of immune function [2], the related transcription factor genes c-myc and c-myb were significantly down-regulated at the time of the exams [3]. Similarly, Morita et al. probed blood samples from graduate students two hours before and two and 24 hours after their oral defense for their Ph.D. thesis and found stress related changes in gene expression, including up-regulation of several interleukins and their receptors [4].

Reduced stress has also been associated with the regulation of gene expression. For example, Qu and colleagues demonstrated significant changes in gene expression levels in peripheral blood cells collected from subjects practicing Sudarshan Kriya, a form of yoga that emphasizes rhythmic breathing techniques, compared to a control regimen [5]. In a study more specific to mental techniques aimed at stress reduction, Dusek et al. collected blood samples from experienced meditators and from novices before and after an 8-week meditation training that focused on the relaxation response. This group found significant alterations in gene expression associated with daily relaxation response practices in both novices and experienced meditators, including regulation of genes directing the generation of reactive oxygen species and response to oxidative stress [6]. A recent study assessed rapid genomic changes during one session of relaxation response practice. Bhasin et al. probed blood cells prior to, immediately after, and 15 minutes after listening to a relaxation response-eliciting audio recording and found enhanced expression of genes associated with energy metabolism and telomere maintenance, and reduced expression of genes linked to inflammatory response and stress-related pathways [7].

Despite the successes of gene expression analysis using peripheral blood samples, practical aspects limit the utility of this technique. Firstly, venipuncture is known to be a stress factor that can have a lasting impact for both children and adults [8-17]. The health risks associated with protocols requiring blood sampling, such as risk of infection any time the skin is broken, also limit their implementation. In addition, the cellular heterogeneity of peripheral blood samples must be considered when interpreting observations of differential gene expression. Since peripheral blood samples are composed of mixed cell populations, there must be an accounting for the possibility that variation in gene expression patterns could be traced to variation in the relative proportions of specific blood cell subsets [18]. This issue is particularly relevant in clinical settings involving stress because various forms of stress can alter white blood cell count [19].

Neutrophils are a promising subset of blood cells that can be targeted for gene expression analysis because they are the most abundant white blood cells in humans and exhibit complex changes in gene expression in response to various stimuli [20]. Roy and colleagues took advantage of the fact that neutrophils migrate to wound sites to collect neutrophils

from young men experiencing examination stress [21]. This group used a skin blister model to collect wound site neutrophils for microarray gene expression analysis and found that psychological stress had a significant effect on the neutrophil transcriptome, including increased expression of genes encoding proteins associated with inflammation. The study reported here takes advantage of the fact that salivary neutrophils leave the vasculature to migrate through the gingivae, where they can be collected non-invasively by a mouthwash procedure.

METHODS

Collection of samples

Five healthy men were recruited to provide mouthwash samples with approval from the Institutional Review Board of the California Pacific Medical Center Research Institute. Participants were between the ages of 19 and 24 years old and had no sign of periodontitis and no trouble giving blood safely. They were asked to refrain from using caffeine after midnight the day prior, and eat a prescribed breakfast the morning of testing and brush and floss teeth no later than 6:30 AM. At 10:00 AM, each subject rinsed for 30 seconds and then expectorated two 15 ml aliquots of Hank's Balanced Salt Solution plus 2 mM calcium and 0.4 mM magnesium at pH 7.4 (HBSS) into a 50 ml tube kept on ice. Immediately following the mouthwash procedure, peripheral blood samples were collected using PAXgene Blood RNA Tubes (PreAnalytiX, Hombrechtikon, Switzerland). The blood tubes were kept at room temperature for 2 hours before storage at -80 oC in accordance with the manufacturer's instructions.

Collection of samples

Salivary neutrophils were isolated from the 30 mL of HBSS following an adapted oral rinse protocol originally developed for dental research to collect salivary neutrophils [22] and separated from contaminating epithelial cells by sequential passive filtration through nylon mesh filters. The final filtrate was centrifuged at 800 rcf, 4oC, for 7 min to pellet the purified salivary neutrophils. The supernatant was aspirated and cells resuspended in 1 mL of HBSS. A 10 μL aliquot was removed for cell counting and the remaining sample was transferred to a 1.5 mL centrifuge tube and centrifuged at 800 rcf, 4oC, for 10 min. The final supernatant was aspirated and the cell pellet was fast-frozen on dry ice before storage at -80 oC.

RNA isolation and quantitative real-time reverse-transcription polymerase chain reaction (qPCR)

Total RNA was isolated from cell pellets using the miRNeasy Mini Kit protocol (Qiagen Foster City, CA) and from blood samples using the PAXgene Blood RNA Purification Kit (PreAnalytiX). Quality of total RNA from two salivary neutrophil samples was assessed by determining the RIN using an

Agilent 2100 Bioanalyzer and associated RNA Pico 6000 LabChip Nucleic acid assay (Agilent Technologies, Santa Clara, CA). This method is based on analysis of microcapillary electrophoresis traces and an algorithm that allows for calculation of RNA integrity using a trained artificial neural network by determining the most informative features that can be extracted from the electrophoretic traces out of 100 features identified through signal analysis. The selected features which collectively catch the most information about the integrity levels include the total RNA ratio (ratio of area of ribosomal bands to total area of the electropherogram), the height of the 18S peak, the fast area ratio (ratio of the area in the fast region to the total area of the electropherogram) and the height of the lower marker [23].

Expression levels for specific gene targets from both cell types were determined in parallel using a SYBR Green qPCR analysis service based on RT² qPCR Primer Assays (SABiosciences, Frederick, MD). The first strand cDNA synthesis procedure included both oligo dT and random hexamers as primers to yield unbiased transcripts and a DNA removal buffer to prevent false positive signals due to amplification of genomic contamination. Each sample was assayed in triplicate and external RNA controls were included to verify efficient reverse transcription and lack of enzyme inhibitors and threshold cycles (Ct) were reported [24].

RESULTS

The oral rinse procedure initially yielded a mixture of exfoliated buccal epithelial cells and salivary neutrophils that were subsequently separated by filtration according to their consistent size difference (see Figure 1). The filtration procedure involved sequential filtration through nylon meshes, and produced a 97% pure population of neutrophils with yields of neutrophils ranging from 500-2,000 neutrophils per collection procedure.

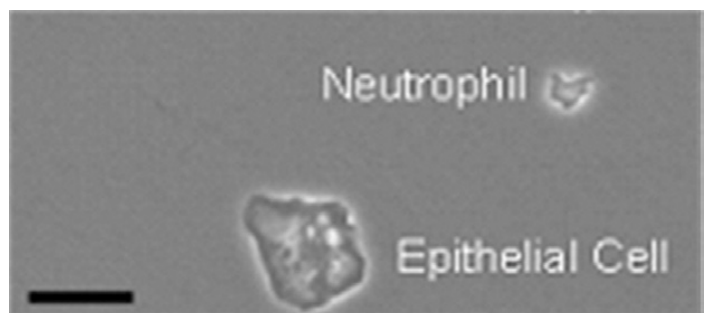**Figure 1. Salivary neutrophils can be separated from contaminating exfoliated buccal epithelial cells due to consistent cell size differences.** Microscopy image shows a salivary neutrophil and exfoliated buccal epithelial cell collected by the oral rinse protocol. Neutrophils are usually 9 to 16 μm in size whereas epithelial cell size ranges from 30 to 60 μm. Cells are shown in phase contrast; bar = 30 microns.

Two salivary neutrophil samples were collected from one participant 40 minutes apart in a pilot experiment to assess the integrity of the RNA harvested from these cells. The total yields of RNA from these samples were 23 ng and 44 ng. As expected for high-quality RNA, the size distribution of RNA fragments generated by electrophoretic separation shows distinct 18S to 28S ribosomal bands (see Figure 2) and the signals for 18S and 28S ribosomal RNA are seen as distinct peaks on electropherograms with minimal evidence of degradation products (see Figure 3). The RIN, a standard integrity measure, determined for these two independent samples were 9.0 and 8.8.

We were interested to compare gene expression values obtained through analysis of salivary neutrophils with those obtained from peripheral blood samples. Samples of both cell types were collected from five healthy subjects and RNA harvested from the two cell sources was analyzed in parallel by qPCR. Four interleukin genes were chosen as targets based on precedent in the literature for their involvement in stress-related signaling pathways: interleukin 1 beta (IL1b), interleukin 8 (IL8), interleukin 10 (IL10), and interleukin 10 receptor beta (IL10Rb). Two genes typically used as reference genes for normalizing target gene values were also analyzed: beta-actin and nicotinamide phosphoribosyltransferase (NAMPT).

As a general quality assessment for the data as a whole, we calculated means, standard deviations (SD), and coefficients of variation (CV) for the triplicate readings of Ct that were well-determined (Ct>30). The variability in readings for one sample from salivary neutrophils and two samples from peripheral blood were somewhat high (CV>.03) and one sample from peripheral blood showed extremely high variability (CV>.15). Review of the values of individual wells revealed that the cause of this variability was due to an inconsistent reading of a single well compared to the other two. The single-well editing criteria was determined such that if one well exceeded the average of the other two wells by one Ct and the difference between the two comparison wells was under 0.2 Ct, then that well was considered an outlier and removed from further analysis. With the exception of these four spurious well readings, the intra-assay variability was nominal for each collection method (mean CV ~ .01).

Regarding expression levels, four of the genes (IL1b, IL8, NAMPT, and beta-actin) were expressed at levels within the range typically found in gene expression analyses in both cell types (Ct<30). Expression levels of IL10 were relatively low compared to all other genes for samples from both cell types (Ct>30) and a significant number of salivary neutrophil samples yielded indeterminate results (Ct>35). Expression for IL10Rb was also low in salivary neutrophil samples (Ct>30).

We next assessed the potential role of NAMPT and beta-actin as reference genes by determining how well they tracked with each other. The difference between their expression levels would be expected to be constant across all samples if they both are suitable for the normalization of the expression data for the target genes. Means, standard deviations, and CVs of the ΔCt between NAMPT and beta-actin were calculated for each collection method (see Table 1). Review of these statistics indicates that both genes are adequate reference genes in peripheral blood but there is considerable variability between these genes in data from salivary neutrophil samples. Since beta-actin had less
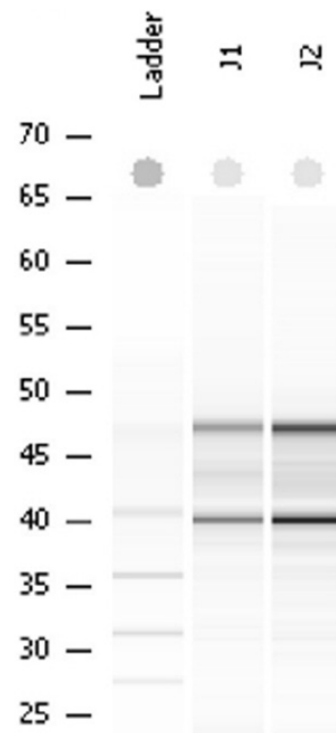
Figure 2.Electrophoretic separation of total RNA harvested from salivary neutrophils reveals distinct 18S and 28S ribosomal RNA bands.
Duplicate samples of total RNA harvested from salivary neutrophils (J1 and J2) were analyzed using the RNA Pico 6000 LabChip Nucleic acid assay and distinct bands for 18S and 28S ribosomal are evident following electrophoretic separation.
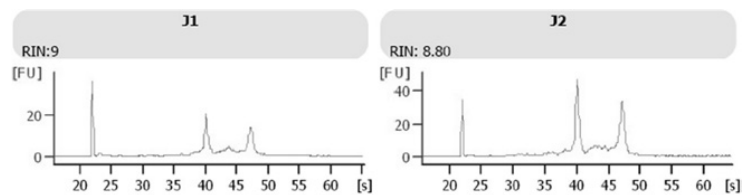
Figure 3.Bioanalyzer profiles of total RNA harvested from of salivary neutrophils show minimal evidence of degradation products.
Electropherograms of total RNA isolated from duplicate samples harvested from salivary neutrophils (J1 and J2) yielded RINs of 9.0 and 8.8.

	Mean	SD	CV
Peripheral Blood	3.59	0.16	0.04
Salivary Neutrophils	1.58	0.97	0.62

Table 1. Statistics on ΔCt of potential reference genes (NAMPT – beta-actin) for each collection method.

variability in the underlying Ct values from salivary neutrophils compared to NAMPT, it was chosen as the single reference gene for subsequent analysis of data from both types of cells.

To compare results between peripheral blood and salivary neutrophils, we calculated the ΔCt for each of the interleukin genes using beta-actin as the reference (values for beta-actin subtracted from target gene values). Examining the ΔCt for each gene across subjects, we observe differences in expression levels as well as the amount of variability between the two types of cell samples (see Figure 4). Three of the target genes (IL1B, IL8, and IL10) appear to be expressed at lower levels than in salivary neutrophils compared to peripheral blood cells. IL10Rb appears to be expressed at very similar levels between the two types of cells. The variability in the data obtained from salivary neutrophils is higher in IL10Rb and IL10, which is to be expected due to the low expression levels. The variability for IL1B and IL8 are much more consistent between the types of cells.
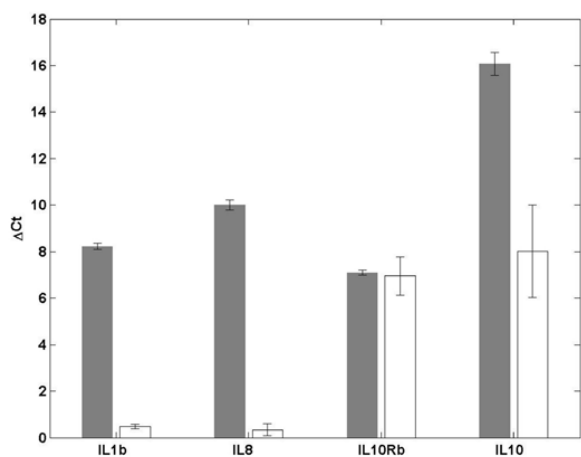
RNA harvested from isolated salivary neutrophils can be probed for target gene expression by qRT-PCR.
The average delta threshold cycles (ΔCt) referenced to beta-actin from five healthy subjects show consistent results within each method of collection (blood: gray bars; salivary neutrophils: white bars). Error bars depict standard error.

DISCUSSION

This study represents the first step in developing a truly non-invasive assay for studying the regulation of human gene expression. The results verify that RNA of sufficient quality for gene expression analyses can be harvested from salivary neutrophils following a mouthwash protocol. The RIN quality assessment method was used because it is independent of sample concentration and analyst, and has become a de facto standard for RNA integrity. The RIN obtained for duplicate RNA samples harvested from salivary neutrophils were 9.0 and 8.8, which falls within the range typically considered appropriate for standard analyses; an RIN higher than five is considered good RNA quality and higher than eight as perfect RNA for downstream PCR applications [25]. Not unexpectedly, we observed differences in expression levels for some of the target genes between salivary neutrophils and peripheral blood. The IL10Rb gene was expressed at similar levels in both cell types but the levels of IL1b, IL8 and IL10

were lower in salivary neutrophils compared to in peripheral blood. These differences in expression levels may be due to multiple causes, including varying RNA stability among cell types and differences related to the handling of the cells during the distinct isolation protocols. Despite these differences, the fact that the variability in the expression values obtained within the cell types is comparable lends credibility to the viability of this method.

Another potential contributor to the variability in expression levels between cell types is differential expression of the target genes within circulating neutrophils compared to salivary neutrophils that have become activated to migrate through the gingivae. While this unique aspect of salivary neutrophils presents a challenge for comparing results with studies using peripheral blood samples, the fact that these cells are the only blood cell subset present in saliva presents the advantage of being able to probe a homogeneous population of blood cells. The resultant decreased signal-noise may provide a more sensitive gene expression assay compared to those based on peripheral blood, due to the cell heterogeneity of peripheral blood samples.

Further characterization is needed to determine optimal conditions for measuring differential gene regulation using the mouthwash protocol, including characterization of the most reliable reference genes for salivary neutrophil analyses. Of the two reference genes evaluated, the beta-actin gene appeared to be more reliable, consistent with a study identifying it as a suitable reference gene for expression studies in human neutrophils using qPCR techniques [26]. However, characterization of the most reliable reference genes for salivary neutrophil analyses will require investigation of an expanded panel. By having a larger set, pair-wise analysis of the variation of expression ratios can be performed to isolate the most robust performers. Additional general guidelines for designing and reporting results of qPCR experiments are published and available to researchers in this field (see http://www.clinchem.org/content/55/4/611.full.pdf+html).

Once optimized, this mouthwash-based assay will allow investigators to utilize the tools of genomics to assess mind-body communication within experimental settings that are not conducive to drawing blood from subjects. The field of therapeutic hypnosis would benefit greatly, for example, as it is poised to make strides in the understanding of emerging pathways of psychosocial and cultural genomics research [27] but introducing venipuncture into protocols could be disruptive. A mouthwash-based assay would also be advantageous for gene expression studies more broadly by simply making subject recruitment easier, since subjects prefer saliva sample collection over blood or urine collection [28], and by eliminating logistical complexities such as the need to catheterize subjects for time series collection. Thus, the development of this assay is timely as genomic data are increasingly being integrated into medicine and hold tremendous promise for providing answers to how aspects of mind might directly influence health and healing.

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THE CREATIVE PSYCHOSOCIAL AND CULTURAL GENOMIC HEALING EXPERIENCE:
A NEW TOP-DOWN EPIGENOMIC PSYCHOTHERAPEUTIC PROTOCOL

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ABSTRACT

This study explores a new top-down epigenomic psycho-therapeutic protocol, the Creative Psychosocial Genomic Healing Experience (CPGHE), for facilitating evidence-based mind-body research in psychology, psychiatry, rehabilitation, therapeutic hypnosis and translational medicine. We experimentally document how the SNCA gene, which codes for the alpha-synuclein protein, was highly and stably down-regulated in lymphocytes of the human peripheral immune system within 1 and 24 hours after treatment with the CPGHE. Dysfunctions of SNCA gene expression are implicated in autism, schizophrenia, Parkinson's, Altzheimer's, alcoholism and a variety of stress related problems. On the positive side the closely related isoform of SNCA, the SNCB gene, is implicated in brain plasticity. We propose that ENCODE – The Encyclopedia of DNA Elements – is the complex adaptive system modulated by the CPGHE. We recommend further research to confirm how the RNA/DNA bioinformatics of the CPGHE could supplement the traditional cognitive-behavioral dynamics of psychotherapy for ameliorating stress related dysfunctions of aging.

INTRODUCTION

Epigenetics is a scientific approach for exploring the inter-action of nature and nurture: how genes interact with the environment to modulate behavior, cognition and consciousness in sickness and health (Feinberg, 2007). Recent research in social psychology has demonstrated how complex epigenetic mechanisms modulate gene expression without altering the DNA code. Epigenetics focuses on a special class of genes described as activity or experience-dependent genes, which can be turned on (activated) or off by signals from the physical and psychosocial environment to modulate the complex human functions of physiology, psychology and consciousness itself (Rossi, 1986, 1993, 2002, 2004, 2007, 2012; Rossi and Rossi, 2013; Lloyd and Rossi, 1992, 2008).
In the past decade DNA microarray technology has made it possible to measure the expression levels of many thousands of genes simultaneously. This novel experimental approach has revolutionized research in molecular biology and become a new standard in personalized medicine (Eisen et al. 1998). Recent research has documented the use of DNA microarrays

for assessing therapeutic responses to psychological relaxation and meditative practices on the molecular-genomic level (Creswell et al., 2012; Dusek et al., 2008; Lichtenberg et al., 2000, 2004), psychosocial variables (Cole, 2009; Cole et al. 2005, 2007, 2010, 2011) We extend the use of DNA microarrays to explore the hypothesis that The Creative Psychosocial Genomic Healing Experience (CPGHE) and related top-down psychobiological processes can modulate gene expression on the molecular-genomic level for reducing symptoms of the stress related disorders (Bar-Joseph et al., 2012: Rossi, Erickson-Klein and Rossi, 2008-2012; Unternaehrer et al., 2012). A full description for the administration and scoring of the CPGHE protocol originally developed by Rossi is freely available (Rossi et al., 2008, Atkinson et al. 2010; Rossi, 2012). We propose that the CPGHE protocol could update the cognitive-behavioral efficacy of evidence-based psychotherapies recommended as the standard of clinical excellence by Insel, (2009, 2010), director of NIMH.

MATERIALS, METHODOLOGY AND SAMPLE DESCRIPTION

The experimental design of this research is essentially identical with our 1st "Pilot Study of Positive Expectations and Focused Attention via a New Protocol for therapeutic Hypnosis Aassessed with DNA Microarrays" (Rossi, Iannotti, Cozzolino, et al. 2008), which had only three subjects. Our current study contains 21 human subjects between 30 and 50 years old (10 male, 11 female), which were randomly selected from the University of Salerno environment in Italy through an interview and a general psychological evaluation made by one of the co-authors (Giovanna Celia) The methodology and DNA Microarrays analysis of this group of 21 subjects as reported here was adapted from its original presentation by Cozzolino, Tagliaferri, Castiglione, Celia et al. (2013).
Approximately 10 mL of peripheral blood was collected from the 21 subjects just before the CPGHE was administered, 1 hour after, and 24 hours after. Blood was processed immediately after collection in order to reduce ex vivo effects of altering the gene-expression profile. Leukocytes of the adaptive immune system were isolated from 5.0 mL whole blood according to the QIAamp RNA Blood Mini manual (Qiagen, Milano Italy). Briefly: erythrocytes were selectively lysed in erythrocyte lysis buffer (EL; Qiagen) by incubation on ice

and vortexing. Leukocytes were pelleted by centrifugation at 400 x g for 10 minutes at 4°C, washed twice with EL buffer and then resuspended in 0.6 ml RLT buffer (Qiagen) containing β-mercaptoethanol. Homogenized cell lysates were finally stored at -80°C until use. The frozen lysates were thawed in a water bath at 37°C until complete dissolving of the saline buffer. Total RNA was extracted using spin columns (Qiagen). Contaminating DNA was removed from total RNA, while was bound to the QIAamp membrane, by means of incubation with DNAse I (Qiagen) following the manual instructions. After DNA digestion, washing and elution of RNA were performed according to the protocol available with the purification kit. RNA concentration was determined using the Picodrop Spectrophotometer apparatus (Saffron Walden, United Kingdom) and its quality assessed on agarose gel electrophoresis.

OUR cDNA SAMPLE PREPARATION AND GENE CHIP HYBRIDIZATION

Single strand biotinylated cDNA was generated as follows: 100ng of total RNA were subjected to two cycles of cDNA synthesis with the Ambion WT expression Kit (Applera, Milano, Italy). The first cycle – first strand synthesis is performed using an engineered set of random primers that exclude rRNA-matching sequences and include the T7 promoter sequences. After second-strand synthesis, the resulting cDNA is in vitro transcribed with the T7 RNA polymerase to generate a cRNA. This cRNA is subjected to a second cycle – first strand synthesis in the presence of dUTP in a fixed ratio relative to dTTP. Single strand cDNA is then purified and fragmented with a mixture of uracil DNA glycosylase and apurinic/apirimidinic endonuclease 1 (Affymetrix Italia, Milano, Italy) in correspondence of incorporated dUTPs. DNA fragments are then terminally labelled by terminal deoxynucleotidyl transferase (Affymetrix) with biotin. The biotinylated DNA was hybridized to the Human Genechip Gene 1ST Arrays (Affymetrix), containing almost 29000 genes selected from H. sapiens genome databases RefSeq, ENSEMBL and GenBank. Chips were washed and scanned on the Affymetrix Complete GeneChip Instrument System, generating digitized image data (DAT) files.

GENE ONTOLOGY: BAYESIAN STATISTICAL INFERENCE AND INTERPRETATION OF GENE EXPRESSION DATA

The Gene Ontology Project (http://www.geneontology.org/GO.doc.shtml#control) provides a set of defined terms for the interpretation of gene expression data. Gene ontology integrates three domains of bioinformatics, how information flows in all life processes: (1) The cellular component, the parts of a cell and its epigenetic environment; (2) The molecular functions, the basic activities of gene expression such as proteins on the molecular level; and (3) complex adaptive systems such as hormonal and immune processes integrating cells, tissues, organs, and organisms (Holland, 2012). Raw data were imported into Bioconductor R and pre-processed using the AFFY library

(Software manual is available from http://cobra20.fhcrc.org/packages/release/bioc/html/affy.html). Different quality control checks such as array images, box plots and relative log expression, were executed before the pre-processing. Robust Multi-array Average (RMA) proposed by Irizarray et al. (2003). Biostatistics 4, 249-264, was used for normalizing data and for obtaining expression measures from Affymetrix .CEL files. Differential gene expression analysis, for the comparisons after 1hour and 24 hours of administrating the CPGHE was done with the LIMMA (Linear Models for Microarray Data) library (software manual available from http://bioinf.wehi.edu.au/limma/). Bayesian statistical methods (Gopnik, 2012; McGrayne, 2011) were used for estimating log-fold changes with this software. Another popular software for evaluating Bayesian methods in evaluating probability rating scales as used in the CPGHE is WinBUGS (Lunn et al., 2000) located at http://www.mrc-bsu.cam.ac.uk/bugs/welcome.shtml. Bayesian inference is particularly appropriate for answering questions such as "What are the alternative states of nature, and how much do I believe they're true?" (McGrayne, 2011, page 209), which is the essential question we are asking about the networks of gene expression that are modulated by the CPGHE (Lunn et al., 2011; Rossi, 2012).

Utilizing Bayesian inference with LIMMA, a false discovery rate (FDR) was carried out to adjust p-values for multiple testing. Genes then were ranked according to Bayesian statistics (essentially the log-odds of differential expression). Genes identified as having a fold-change of 1.2 and an adjusted p-value threshold of 0.05 were considered to be significantly differentially expressed. To investigate the biological meaning of this large list of significant genes, we used DAVID (http://david.abcc.ncifcrf.gov/) - a set of functional annotation tools to identify enriched gene ontology (GO) terms and well-known KEGG and BioCarta pathways. For each DAVID result an adjusted p-value representing the level of significance of that GO term or pathway is given, therefore we selected only those items having an adjusted p-value less than 0.05.

RESULTS: GENE ONTOLOGY (GO) ANALYSIS OF GENE EXPRESSION MODULATED BY THE CPGHE

Figure 1 illustrates our gene ontology (GO) analysis of gene expression modulated by the CPGHE in the peripheral blood mononuclear cells (PBMCs) of our subjects. We found 36 genes were de-regulated in both groups (Group B, 1h and Group C, 24h). We established 3 categories of genes: early genes (8 down-regulated and 2 up-regulated) belonging only to group B, late genes (88 down-regulated and 30 up-regulated) belonging only to group C; and early/late genes (34 down-regulated and 2 up-regulated) present in both group B and group C.

The biological Profiling of Gene Groups utilizing Gene Ontology analysis segregated these groups of genes into 15 biological pathways (GO:0005833: hemoglobin complex and blood group antigen, 5 and 6 genes, respectively; GO:0030863: cortical cytoskeleton, 7 genes; GO:0044445: cytosolic part, 9 genes; GO:0043066, GO:0043069, GO:0060548, negative regulation of apoptosis, negative regulation of programmed cell death, negative regulation of cell death, 13 genes, respectively; GO:0044448, cell cortex part, 7 genes; GO:0042981, GO:0043067, GO:0010941, regulation of apoptosis, regulation

of programmed cell death, regulation of cell death, 20 genes, respectively; GO:000582, cytosol, 27 genes; Acetilation, 39 genes; GO:0005938, cellcortex, 8 genes; GO:0006916, anti-apoptosis, 9 genes; h_ahsp Pathway:Hemoglobin's Chaper-one, 4 genes).

The more frequently represented biological pathway was related to regulation of apoptosis: GO:0043066, GO:0043069, GO:0060548, negative regulation, 13 genes; GO:0042981, GO:0043067, GO:0010941, regulation of apoptosis, 20 genes and GO:0006916, antiapoptosis, 9 genes. Multiple relevant biological processes, such as: "antiapoptotic genes" GO:0006916, SNCA, BCL2L1, ALSCR2, BMIP3L (early/late responsive genes), PIM2, RELA, UBB, CFL1, MYD88 (late genes); "negative regulation of apoptosis", GO:0043066, GO:0043069, GO:0060548, CSDA (early/late responsive genes), PIM1, NRAS, BCL3 (late responsive genes); "regulation of apoptosis genes", GO:0042981, GO:0043067, GO:0010941, IKBKG, PPP3R1, TP53INP1, ING4, IL2RA, TRADD, FEM1B (late responsive genes), were found as illustrated in Figure 2.

This analysis documented that only the SNCA gene transcript was down-regulated with highest logFold change value (-2.5) documented in Figure two. The down-regulation observed in the other genes of the same biological pathway, had a logFold change value about -0.5/-1, suggesting that the psy-chobiological effects of the "CPGHE treatment" may facilitate cellular homeostasis to achieve this effect.

The SNCA gene encodes a small protein called alpha-synu-clein. Alpha-synuclein is abundant in the brain, and smaller amounts are found in the heart, muscles, and other tissues, such as in peripheral blood including mononuclear cells (Shin et al., 2000) and is secreted into plasma (El-Agnaf et al., 2003). In the brain, alpha-synuclein is found mainly at the tips of neurons in specialized structures called presynaptic termi-nals. Although the function of alpha-synuclein is not well understood, studies suggest that it plays an important role in maintaining a supply of synaptic vesicles in presynaptic terminals. It may also help regulate the release of dopamine, a type of neurotransmitter that is critical for controlling the start and stop of voluntary and involuntary movements.

At least 18 mutations in the SNCA gene have been found associated with Parkinson's disease (PD, MIM168600), a neurodegenerative disease characterized by akinesia, rigid-ity, tremor, and postural instability a condition character-ized by progressive problems with movement and balance (Nuytemans et al., 2010; Coppedè, 2012). It is unclear how alterations in SNCA gene expression are related to Parkinson disease, but it is known that misfolded or excess alpha-synu-clein proteins may cluster together and impair the regula-tion of dopamine in specific regions of the brain. The excess alpha-synuclein protein is related to increased expression of SNCA mRNA levels in dopaminergic neurons (Maraganore et al., 2006; Grűndemann et al., 2008). The loss of dopamine regulation weakens communication between the brain and muscles untithe brain cannot control muscle movement (Spillantini et al., 1997; Braak et al., 2003).

Beyond the regulatory DNA sequence, several additional levels of epigenetic transcriptional control have become ap-parent recently on SNCA gene expression control (Suzuki and Bird, 2008).
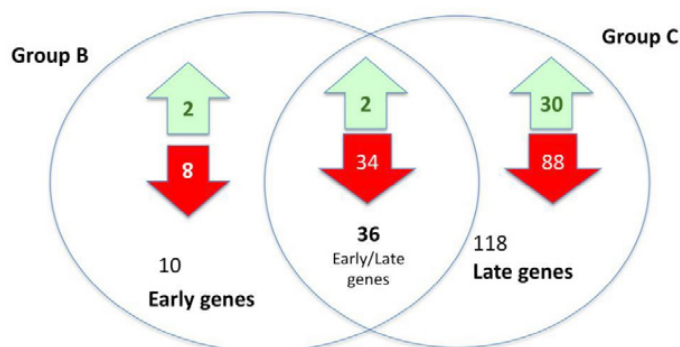
Figure 1.Responsive genes in PBMCs of our samples
Immediate-early responsive genes belonging only to GroupB, late responsive genes are present only GroupC, early/late genes are in common between GroupB and GroupC.
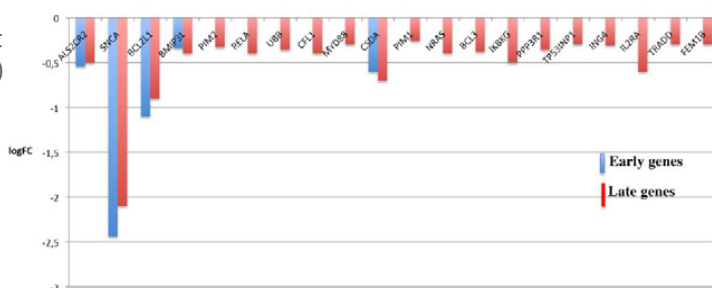
Figure 2.Responsive genes in PBMCs related to regulation of apoptosis
The regulated genes in the GO apoptosis regulation are shown.
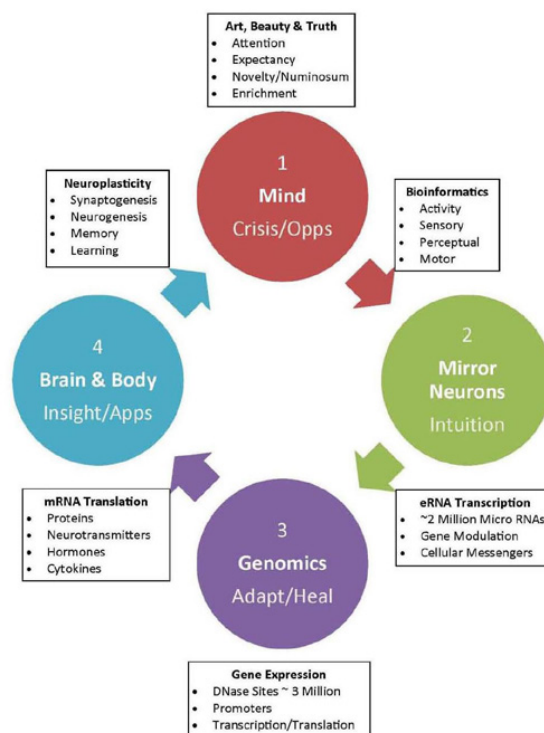
Figure 3.
This is our proposal for a very broad functional definition of the gene as a complex adaptive system that embraces the daily and hourly cycles of life processes on all levels from the mind to DNA (Lloyd & Rossi, 1992, 2008). We propose that research on these four major dimensions of The Classical Psychosocial Epigenome: Mind, Mirror Neurons, Genes and the Brain/Body underpin the psychobiological basis of the Creative Psychosocial Genomic Healing Experience (CPGH).

The SNCA gene has two CpG islands and their methylation decreased gene expression, while inhibition of DNA methylation activated SNCA expression. Methylation of CpG dinucleotides is a prime epigenetic mechanism and a frequent biochemical modification of DNA in the human genome. Aberrant DNA methylation of SNCA gene has also been associated with psychiatric conditions and might constitute a pathogenic mechanism for other diseases as well (Feinberg, 2007).

Recently the microarray-based transcriptome analyse of peripheral blood from patients with malignancies, infectious diseases, autoimmunity and cardiovascular diseases (Chaussabel et al., 2010; Staratschek-Jox et al., 2009) are able to identify biomarkers used as an indicator of a normal biologic process, a pathogenic process, or a pharmacologic response to a therapeutic agent. Studies in individuals with alcoholism (Bönsch et al., 2005) and anorexia patients (Frieling et al., 2007) documented hypermethylation of the SNCA promoter, suggesting how this gene's expression can be modulated epigenetically. It is considered the top candidate gene and blood biomarker in increased alcohol consumption (Foroud et al., 2007). Publicly avaliable data bases implies how dysfunctions of SNCA gene expression is implicated in autism (Buxbaum et al., 2004), schizophrenia (Paunio et al., 2004), Parkinson's, Altzheimer's, alcoholism (Reich et al., 1998) and a variety of other stress related problems associated with post-traumatic stress disorder (Neylan et al., 2011).

It is interesting to note that the U.S. Patent office granted a patent on a molecular-genomic agent for inhibiting alpha-synuclein (SNCA) gene expression for the treatment of neurodegenerative disorders (Bumcrot, 2009). Further research by independent research groups is now required to confirm how the molecular-genomic dynamics of the CPGHE could supplement translational medicine as well as the traditional cognitive-behavioral dynamics of psychotherapy for ameliorating the ageing and stress related dysfunctions. For a broader perspective on the significance and implications of the CPGHE for therapeutic hypnosis, psychotherapy and the holistic healing arts of translational medicine we base our evolving epigenetic concept of the psychosocial genome on ENCODE – The Encyclopedia of DNA Elements (Rossi & Rossi, 2013).

ENCODE RESEARCH AND THE EPIGENOMICS OF THE PSYCHOSOCIAL AND CULTURAL GENOME

A major motivation for focusing on the epigenetic molecular-genomics of the therapeutic CPGHE protocol is the recent publication of 30 leading papers about ENCODE – The Encyclopedia of DNA Elements – in major scientific journals such as Nature, Science Genome Research and Genome Biology. We propose that ENCODE is a major international research organization that is potentially capable of integrating the mind-body psychobiological basis of the holistic healing arts. The ENCODE consortium introduced the deep biological significance of its research in this way (ENCODE project consortium, 2012).

"The human genome encodes the blueprint of life, but the function of the vast majority of its nearly three billion bases is unknown. The Encyclopedia of DNA Elements (ENCODE) project has systematically mapped regions of transcription, transcription factor association, chromatin structure and histone modification. These data enabled us to assign biochemical functions for 80% of the genome, in particular outside of the well-studied protein-coding regions. Many discovered candidate regulatory elements are physically associated with one another and with expressed genes, providing new insights into the mechanisms of gene regulation. The newly identified elements also show a statistical correspondence to sequence variants linked to human disease, and can thereby guide interpretation of this variation. Overall, the project provides new insights into the organization and regulation of our genes and genome, and is an expansive resource of functional annotations for biomedical research.

The human genome sequence provides the underlying code for human biology. Despite intensive study, especially in identifying protein-coding genes, our understanding of the genome is far from complete, particularly with regard to non-coding RNAs, alternatively spliced transcripts and regulatory sequences. Systematic analyses of transcripts and regulatory information are essential for the identification of genes and regulatory regions, and are an important resource for the study of human biology and disease. Such analyses can also provide comprehensive views of the organization and variability of genes and regulatory information across cellular contexts, species and individuals.

The ENCODE project aims to delineate all functional elements encoded in the human genome. Operationally, we define a functional element as a discrete genome segment that encodes a defined product (for example, protein or non-coding RNA) or displays a reproducible biochemical signature …

The advent of more powerful DNA sequencing technologies now enables whole-genome and more precise analyses with a broad repertoire of functional assays. (p. 57-74, Ital added here) "

Notice how this very significant historical statement by ENCODE is only about the biological bottoms-up approach to understanding human nature. The ENCODE consortium does not discuss the top-down approach of psychosocial genomics – how mind and consciousness apparently can modulate its own functional phenotypic expression (observable behavior). It is, however, the top-down perspective from mind to gene that we seek to complement the traditional bottoms-up perspective of molecular biology in its applications to psychology, psychiatry, psychotherapy and translational medicine as illustrated in figure three with the CPGHE.

----------------PHOTO 3-----------------

Much of the ENCODE research implies the need for a new bioinformatic conception genes and their epigenetic relationships to the environment as complex adaptive systems (Holland, 2012). Figure 3 is our proposal for a functional definition of the gene as a complex adaptive system that embraces the entire daily and hourly Basic Rest-Activity Cycle (BRAC) of life processes on all levels from the mind to gene and molecules (Lloyd and Rossi, 1992, 2008). We propose that research on these 4 major levels of The Psychosocial Genome: Mind, Mirror Neurons, Genes and the Brain/Body illustrated in figure 3 can be conceptualized as a complex adaptive system as follows.

1. Mind-Body Research in the top circle embraces the classical experimental research of historical psychology (Boring, 1950) with the addition of the more recent emphasis on consciousness studies of art, beauty, creativity, music, truth, dreams, meditation and imagination in current neuroscience and psychosocial genomics (Rossi, 1972/2000, 2002, 2004, 2007, 2012). Key research at this top level of consciousness, dreaming, and imagination explores the Novelty-Numinosum-Neurogenesis-Effect as a complex motivational adaptive system, which integrates gene ontology with brain plasticity and the holistic healing arts of psychotherapy and translational medicine illustrated in this and related papers (Cole et al., 2007-2011; Creswell et al., 2012; Dusek et al., 2008; Lichtenberg et al., 2000, 2004; Rossi, Iannotti, Cozzolino et al., 2008).

2. Mirror Neuron Research initiated by Rizzolatti, Gallese (Rizzolatti & Sinigaglia, 2008) and others at the University of Parma in Italy has been greatly expanded in this paper from its original neural level focus in the specific F5 area of the brain. Key research now explores the bioinformatic molecular levels of cellular signaling conceptualizing consciousness as a complex adaptive system integrating information (Tononi, 2008, 2012) from mind to gene via eRNAs, mRNAs, etc., throughout the brain and body (Iacoboni, 2008; Rossi, 2002, 20004, 2007, 2012).

3. Genomic Research via the ENCODE project that includes activity and experience-dependent gene expression is currently manifesting a profound breakout on the epigenetic level. Key research is now exploring complex adaptive systems of information transduction in the transcription process arising from ~2 million eRNAs carrying signals from the physical environment and psychosocial milieus to genes bearing ~3 million docking sites recently summarized by the ENCODE Consortium (2012).

4. Mind-Brain-Body Consciousness Research has a new psychobiological foundation in the transcription/translational process of coding cognition with eRNAs (ExRNAs, mRNAs etc.) and proteins at the molecular-genomic level. Key research explores how these proteins, often called "mother molecules," are cleaved into the neurotransmitters, hormones, and cytokines of the complex adaptive systems of psychoneuroimmunology, which integrates molecular epigenomics at the cellular level of the brain and body. This illustrates the molecular bioinformatics that ultimately underpins the dynamics of memory, learning, behavior with the qualia of consciousness and cognition via synaptogenesis and neuroplasticity in the neuroscience of psychotherapy and translational medicine (Rossi, 2012; Rossi and Rossi, 2013).

DISCUSSION

This exploratory research was undertaken to determine whether the Creative Psychosocial Genomic Healing Experience (CPGHE) on the cognitive-behavioral level could modulate the transcription/translational dynamics of gene expression at the cellular level. Our major unexpected finding that the SNCA gene was deregulated within human peripheral blood lymphocytes motivates this discussion of its possible implications for human health and the epigenomics of consciousness research.

This surprising result must now be replicated by other research groups with larger human clinical populations and more extensive controls to achieve scientific reliability and validity. Our results receives some credibility from more recent research (Li-Huei & Madabhushi, 2014; Lu et al., 2014) that suggests how a number of molecular mechanisms at the epigenomic level could modulate gene expression associated with cognitive decline and neurodegeneration in Altzheimer's disease during cellular stress and aging as follows.
In a recent abstract of their paper Lu et al. (2014) introduce "REST and stress resistance in ageing and Altzheimer's disease" in this way.

"Human neurons are functional over an entire lifetime, yet the mechanisms that preserve function and protect against neurodegeneration during ageing are unknown. Here we show that induction of the repressor element 1-silencing transcription factor (REST; also known as neuron-restrictive silencer factor, NRSF) is a universal feature of normal ageing in human cortical and hippocampal neurons. REST is lost, however, in mild cognitive impairment and Alzheimer's disease. Chromatin immunoprecipitation with deep sequencing and expression analysis show that REST represses genes that promote cell death and Alzheimer's disease pathology, and induces the expression of stress response genes. Moreover, REST potently protects neurons from oxidative stress and amyloid β-protein toxicity, and conditional deletion of REST in the mouse brain leads to age-related neurodegeneration. A functional orthologue of REST, Caenorhabditis elegans SPR-4, also protects against oxidative stress and amyloid β-protein toxicity. During normal ageing, REST is induced in part by cell non-autonomous Wnt signalling. However, in Alzheimer's disease, frontotemporal dementia and dementia with Lewy bodies, REST is lost from the nucleus and appears in autophagosomes together with pathological misfolded proteins. Finally, REST levels during ageing are closely correlated with cognitive preservation and longevity. Thus, the activation state of REST may distinguish neuroprotection from neurodegeneration in the ageing brain."

In an important commentary on this Lu et al. (2014) paper Li-Huei Tsai & Ram Madabhushi (2014) discuss REST as "A protective factor for the ageing brain" as follows.

In their study, Lu et al. used a dazzling array of experimental approaches to demonstrate that the loss of neuroprotective REST functions contributes to neuronal vulnerability in the brains of those with Alzheimer's. The authors observed that, in the brains of healthy aged individuals, nuclear REST both targets and suppresses several pro-apoptotic genes, as well as certain genes that encode enzymes involved in the pathology of Alzheimer's. But in diseased brains this suppression is lost, resulting in the induction of genes that are likely to underlie aspects of neuronal loss and neurodegeneration (Fig. 4).
Excitingly, this study provides the first detailed investigation of molecular markers in the brain that differentiate between populations of the young, the aged and those with Alzheimer's. Furthermore, by showing that ageing in the brain might be associated with the activation of a specific stress-response program, it implies that sustained maintenance of this program confers protection from neurodegeneration. Indeed, all the healthy centenarians studied in the research showed uniformly high levels of REST.
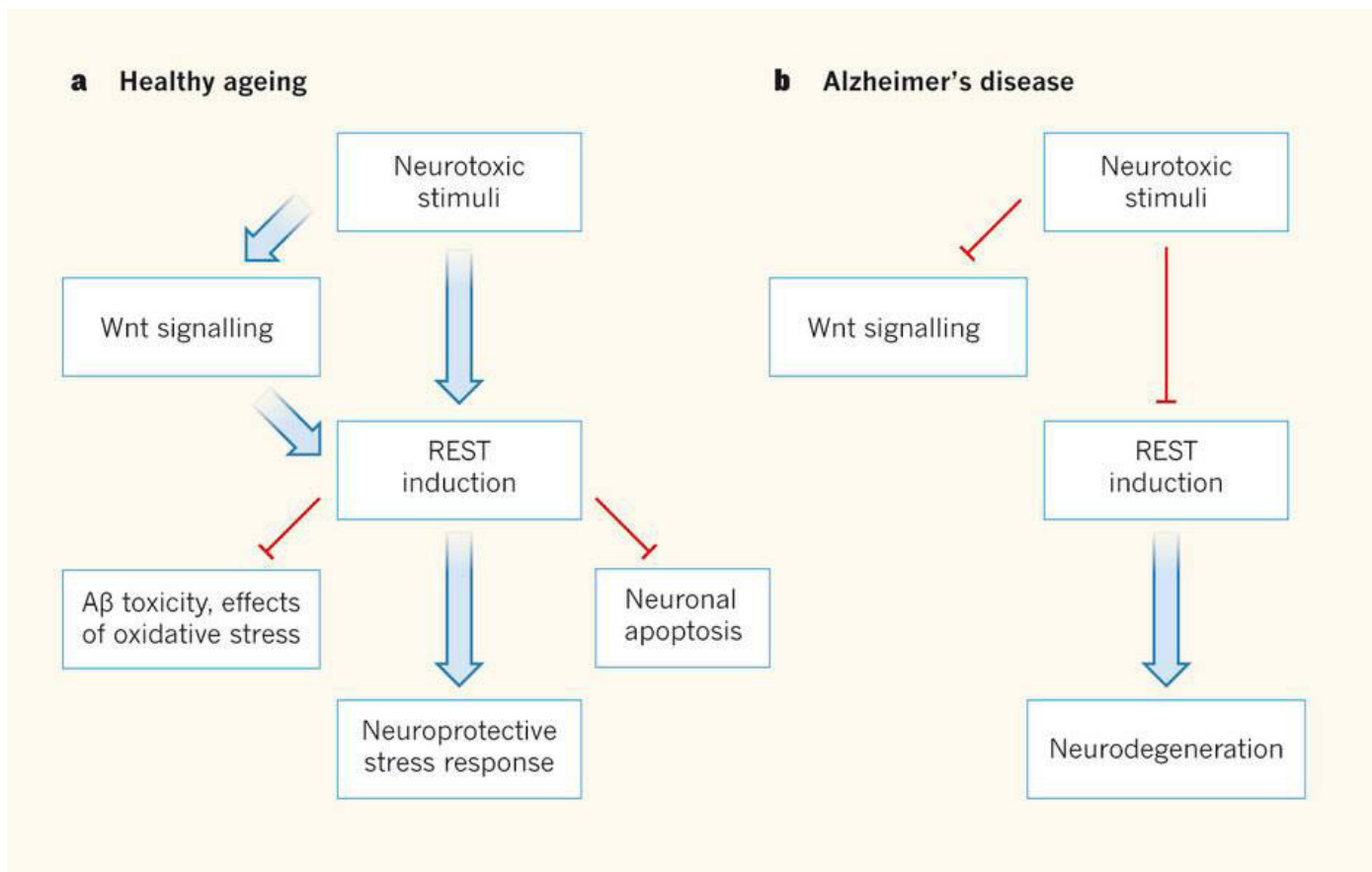
Figure 4.
REST activation distinguishes healthy aged brains from the brains of those with Alzheimer's disease. a. Lu et al. (2014) report that various neurotoxic stimuli in the ageing brains of healthy people cause increased expression of the REST protein, an effect that is at least partially dependent on the Wnt-signalling pathway. REST induction activates neuroprotective stress-response pathways, while suppressing neuronal apoptotic cell death and the toxic effects of the accumulation of amyloid-β (Aβ, the main protein component of the plaques found in the brains of patients with Alzheimer's disease) and of elevated oxidative stress. b. In the brains of those with Alzheimer's disease, both Wnt-signalling and REST induction are suppressed, leading to neurodegeneration. (Nature, 2014, 507, 448-454)

Could therapeutically stimulating nuclear REST activity in the brain prevent Alzheimer's and other degenerative diseases? On the basis of Lu and co-workers' results, one strategy would be to activate Wnt signalling in aged individuals. However, such activation is also implicated in the development of various cancers, and so this approach would probably require careful targeting of Wnt activation in the brain. Alternative strategies include finding either Wnt-independent REST activators or small molecules that prevent the export of REST from the nucleus. A deeper understanding of the molecular mechanisms that govern REST activation in the ageing brain will be crucial for such efforts to be successful." (Nature, 2014, 507, 1-2)

The unexpected and surprising results of the down regulation of the SNCA gene in our research as a result of the application of the psychosocial and cultural epigenomics of the Creative Psychosocial Genomic Healing Experience (CPGHE) protocol for stress reduction (Cozzolino et al., 2013) in association with the more recent research of Lu et al. (2014) and the highly relevant comments of Li-Huei Tsai & Ram Madabhushi (2014) suggest that the molecular dynamics of Wnt signaling and cellular stress in Alzheimer's disease illustrated in figure 4 imply that a new top-down approach to consciousness and cognition research may now be at hand for optimizing psychotherapy and translational medicine.

SUMMARY AND CONCLUSIONS

aThe exploratory research of this paper identified how the SNCA gene was highly and stably de-regulated in the peripheral blood lymphocytes of the immune system of human subjects within 1 and 24 hours after administration of the Creative Psychosocial Genomic Healing Experience, a cognitive-behavioral approach to psychotherapy, rehabilitation and translational medicine. Dysfunctions of the SNCA gene are known to be associated with autism, schizophrenia, Parkinson disease, Alzheimer's disease, alcoholism, aging and other stress related dysfunctions. On the other hand the closely related isoform of the SNCA gene, the SNCB gene, is implicated in healthy brain plasticity, memory, learning and behavior. Further research with larger clinical populations and more comprehensive controls is now required to confirm how therapeutic psychosocial and cultural processes associated with administration of The Creative Psychosocial Genomic Healing Experience may modulate complex adaptive epigenomic systems in stress related dysfunctions during psychotherapy and translational medicine.

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THERAPY:

NO IMPROVEMENT FOR 40 YEARS

John Thomas

ANAHEIM

The good news is that psychotherapy continues to help around 80 percent of the people who seek the assistance of therapists to deal with their problems.

The bad news is that psychotherapy has not shown any improvement during the last 40 years in how well therapistsdeliver their services.

The good news/bad news scenario was delivered by Scott Miller, Ph.D., to several hundred therapists attending his workshop at the quadrennial Evolution of Psychotherapy conference, which attracted nearly 8,000 psychologists, psychiatrists and other therapists in December. It is sponsored by the Milton H. Erickson Foundation, California State University Fullerton and California Southern University.

Those attending Miller's entertaining three-hour lecture were seeking ways to avoid becoming average therapistsand enhance their performance and join the group of so-called top performers who achieve about 50 percent better outcomes than their equally trained and credentialed peers.

Over the last decade, Miller and his colleagues at the International Center for Clinical Excellence in Chicago have been tracking the outcomes of thousands of clinicians around the world to identify the practices that make them well above average.

Miller said practice for most clinicians is "like riding an exercise bike. We work up a sweat, but we don't get anywhere." While psychology as a profession seems stuck in the 1970s in terms of its effectiveness, athletic performances have increased by more than 50 percent during the same period of time. Available evidence, he added, demonstrates that attending a typical continuing education workshop, specializing in the treatment of a particular problem or learning a new treatment model does little to improve therapeutic effectiveness. And despite what many psychologists and other therapists think, there isn't any evidence that they get better over their careers.

Client dropout from therapy continues at around 47 percent, Miller said, and every tenth person in therapy leaves worse off than when they started. There is little difference in outcomes, whether therapy is provided by experienced professionals, students, early career practitioners or paraprofessionals.

When he asked for suggestions from the audience as to why psychology is stuck in the 1970s in terms of its effectiveness, responses generally challenged the findings that Miller presented, including polling error and misinterpretation of data.

"And I thought it was only your clients who are in denial," he responded, to a roomful of laughter.

Generally, the top performers in psychotherapy know more, see more and do more than psychologists and other therapists who never leave the ranks of average, Miller said. But, how they do that involves time and effort many therapists aren't willing to undertake, despite their desires to do better than average.

Miller used two examples to impress upon his audience the single most important element in becoming a top performer.

First he asked for a show of hands on a short series of questions concerning championship chess players. Two of the answer choices dealt with the amount of time a chess player devotes to playing the game, while the third involved reading books about various proven chess strategies.

Almost everyone chose the answers dealing with the amount of time champions spent playing chess. It appeared only one person chose the third choice that involved reading about chess, which was the correct response.

"You can't do better therapy by attending workshops and you can't improve your therapy skills while doing therapy," Miller said.

His second example was an 8-yearold girl who played a piano so well some of her listeners thought it was a recording. Miller learned from the girl that her skills were developed over a few years by practicing the piano four hours a day every day.

"The only two days she didn't practice was on Christmas and her birthday," Miller explained. The girl also told Miller that if she was having trouble getting through a tough spot on the piece she was playing, she would concentrate solely on that piece until she got it right.

"This is what top performers in therapy do. They spend a great amount of time constantly going over their mistakes until they get it right," Miller said. Top performers spend a lot of time getting ready for therapy sessions and a lot of time afterward

critiquing their performances. Many rely on being observed by highly regarded colleagues to see if they are doing well. Average therapists can also improve their performance by adopting Feedback Informed Treatment (FIT), which allows clients to tell clinicians what they like or dislike about their treatment.

He noted that the Substance Abuse and Mental Health Services Administration has deemed FIT an evidence-based treatment practice. Research conducted at multiple sites across a wide range of clients and presenting complaints indicates that clinicians can improve the outcome of those cases most at risk for failure by as much as 65 percent without changing their preferred treatment approach or learning any new treatment techniques.

While many sessions dealt with the past, one dealt with facilitating the RNA/DNA epigenetics on creating new consciousness as the next step in the evolution of psychotherapy.

Led by Ernest Rossi, Ph.D., and Kathryn Rossi, Ph.D., the workshop on RNA/DNA epigenetics looked more like a course on biology than psychology and experiential treatment sessions looked a lot like Transcendental Meditation.

The goal of Epigenetics Psychology is the practical application of knowledge gained from epigenetic research. The field helps to explain how nurture shapes nature, where nature refers to biological heredity and nurture refers to virtually everything that occurs during the lifespan.

Other sessions featured many of the "big names" of psychotherapy, such as Aaron Beck, M.D.; Martin Seligman, Ph.D.; Salvador Minuchin, M.D.; Steven Frankel, Ph.D., J.D., and Irving Yalom. M.D. Showcasing leaders and pioneers in the field has been the hallmark of the Evolution conferences since the first was held in 1985 in Phoenix, Arizona.

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THE BEGINNER'S MIND
A REVIEW OF THE COLLECTED WORKS OF
MILTON H. ERICKSON

Richard Hill

How do you create another Erickson, or one might also say, a Pavarotti or a da Vinci, inside yourself? Much about Milton H Erickson may well be un-reproducible, but as you read through the 17 volumes of The Collected Works of Milton H Erickson, it is possible to see not only the uniqueness of Erickson, but also the uniqueness that Erickson stimulated in those around him. Jay Haley, a 20 year student of Erickson described him in, Uncommon Therapy as "a standout as a unique school in himself, and the usual premises of psychiatry and psychology were not adequate to describe him." (Haley, 1986, p.11)

Ernest Rossi studied with Erickson during the last eight years of his life. They spent one week together every month. It was in the context of his depth of engagement with Erickson that Ernest Rossi took on the task as one of the editors of these volumes. The editorial team is complete with Roxanna Erickson-Klein, daughter of Erickson and Kathryn Rossi. The task of reviewing these volumes has cheerfully fallen to me as I read every word of every volume, with a beginner's mind.

Many of Erickson's published and unpublished papers are reproduced. Rossi has also included some of the modern neuroscience and psychosocial genomics from recent years that underscore Erickson's extraordinary perceptions. Volume 1 reproduces the papers from the 1920's and 30's that show Erickson's search for an understanding of therapeutic hypnosis. In 1923 he was a beginner, conducting his investigations under the supervision of Clarke C. Hull at the University of Wisconsin. Yet, even as he began his investigations, Erickson strongly felt there was an error in Hull's conviction that the operator was "more important than any inner behavioural processes of the subject" (Vol 1, p. 74). Erickson had a beginner's mind and a strong sense of where to begin. Rossi's beginners mind, although steeped in a history of academic, theoretical expertise, at times seemed at odds with Erickson's spontaneous and reality directed approach. This difference was, however, of great importance. Rossi's investigations found current theoretical developments and research which give both foundation and validity to Erickson's ingenious practices.

An important breakthrough was the discovery of Kleitman's (1969) natural Basic Rest and Activity Cycle (BRAC) which proposed that the 24 hour circadian rhythm could be firther divided into 90-120 minute ultradian cycles. It explained why Erickson intuitively gave long 90-120 minute sessions.

When Rossi asked him why he did this, Erickson replied, "That is how much time it takes to get something done!" Kleitman's research provided a scientific clue to Erickson's "uncommon therapy". This scientific revelation created a lasting bond between Rossi and Erickson. Both were united in the beauty and truth of this epiphany of new understanding.

Erickson was, without doubt, prescient. His research began in the 1920's to understand what therapeutic hypnosis was all about. Through out his life, he teased apart the nature of the process, practice and benefits. The difficulty with prescience, however, is that it is impossible to know at the time if the work will be validated by later scientific research. History has shown that some propositions have proved to be off the mark. Phrenology was prescient in that it embraced the precept that the brain had specific areas for particular activity, but it was certainly not bumps on the head that reflected this. One might even say that Lamarck was onto something when he talked about changes in genetics in response to repeated activity, which is now seen as possible through epigenetics, but giraffes did not grow long necks in a single lifetime any more than my beard will stop growing despite shaving every day.

Among many things that Erickson proposed was the possibility that the mind could influence body functions and, in the right circumstances, generate healing processes. Erickson challenged this first hand when he paddled the Mississippi to regain strength after his first bout of polio (Vol 1, p. xi) and later in how he would distract, displace and reinterpret pain that often wracked his body in later years (Vol 1, pp. 201-208) due to post-polio syndrome. Rossi sought to understand the deeper biology of how this mind-body activity might come about. Psychosocial genomics (Rossi, 2002; Hill, 2012) seeks to explain the nature of activity-dependent-gene-expression as a response to non-invasive therapies like psychotherapy and therapeutic hypnosis. It is fitting that Vol 1 begins with a paper from Rossi that describes the propositions of psychosocial genomics. This gives the reader background knowledge to see the prescience in Erickson's writing and to be, frankly, even more amazed.

These volumes unfold as a true "collection", rather than just a bland chronology. Each volume is dedicated to a particular theme or shift in progress. . Published papers; conference

presentations; transcriptions of recordings of actual sessions, interviews and conversations; anecdotes; and fragments of notes and comments all come together to highlight the particular intention of each volume. Sometimes it is like reading an adventure novel. The drama, the mystery and the suspense created in the case studies and the extraordinary effort as Erickson seeks to tune into the patient to help them find their own healing. At times I was breathless, poised at the end of a page to instantly turn to the next. But, where fiction is organised and an intentional fabrication of reality, for Erickson and his patients this was reality – brazen, brash, unpredictable, yet all the while conducted within the safe containment field of therapeutic hypnosis. This overview review is limited in its scope, but as each volume is reviewed individually, the more recent breakthroughs in neuroscience, biology, genetics, epigenetics and psychosocial genomics will be incorporated to further highlight the prescience of Erickson.

Future reviews of individual volumes will seek to give some insight into the contents, connections to current research and also reveal the highlights as they appear to this beginner's mind. I have already made some mention of Vol 1. Here are just a few of the many highlights that captured my attention in the other 16 volumes:

Vol 1: The Nature of Therapeutic Hypnosis

Vol 2: Basic Hypnotic Induction and Suggestion includes a detailed transcript of a trance induction with commentary and discussion by Haley and Weakland.

Vol 3: Opening the Mind. Erickson, Rossi and Moore (Erickson's personal physician) answer Rossi's question in his last meeting with Erickson: How can I have an open mind? This is an extraordinary written portrayal of the student in the learning embrace of the master.

Vol 4: Advanced Approaches to Therapeutic Hypnosis covers creative, adaptive processes utilized by Erickson. The confusion technique and the use of psychological shock to 'facilitate new identity creation' are among the many intriguing approaches and case studies described.

Vol 5: Classical Hypnotic Phenomena, Part 1 teaches fundamentals of hypnosis from papers published in 1930's and 40', interestingly punctuated by a 1973 conversation between Erickson and Rossi and a 1962 paper co-written with Allan Erickson.

Vol 6: Classical Hypnotic Phenomena, Part 2 expands Erickson's work with other researchers, including Elizabeth Erickson; a chapter by Stephen Langton; and a description of the Psychosocial Genomic Healing Response developed by Ernest Rossi.

Vol 7: Mind Body Healing and Rehabilitation reveals Erickson's pioneering approach to biological dysfunction, pain, rehabilitation, and healing. Rossi describes the current scientific foundation of mind-body healing and rehabilitation including brain plasticity, gene expression and the facilitation of natural internal healing processes, supporting Erickson's intuitive genius, prescience, and continuing relevance.

Vol 8: General and Historical Surveys of Hypnosis offers a historical exposition of therapeutic hypnosis. Erickson's book reviews from the 1960's are truly fascinating, showing his capacity to compliment and praise as well as his erudite capacity to be scathing.

Vol 9: The February Man is the famous case study of The February Man. The explanations and discussions between Erickson and Rossi as they review the transcribed recordings of the sessions are as riveting as they are informative.

Vol 10: Hypnotic Realities: The Induction of Clinical Hypnosis of Indirect Suggestion is another previously published case. Hypnotic Realities is reproduced in its original font and form, but with the valuable addition of an introduction from the editors and a concluding chapter by Ernest and Kathryn Rossi updating the science of suggestion as an implicit processing heuristic.

Vol 11: Hypnotherapy an Exploratory Casebook is the third of four books written by Erickson and Rossi. It is like attending a private masterclass with Erickson - preserved in time on the written page. Transcripts of more than 20 cases give you a direct experience of Erickson's therapeutic practices.

Vol 12: Experiencing Hypnosis is the fourth book by Erickson and Rossi. Rossi describes it as Erickson training Rossi in clinical hypnosis. Transcripts of Erickson's casework are discussed and annotated by Rossi throughout. As Rossi learnt then, we learn now.

Vol 13: Healing in Hypnosis is the first of four volumes that bring together Erickson's seminars, workshops and lectures. Florence Sharp began collecting audio and written records in the 1960's resulting in these fascinating transcripts of Erickson 'live'. The opening chapter is a biographical chapter that not only describes Erickson's upbringing, but also his emergence as a therapist.

Vol 14: Life Reframing in Hypnosis shows how Erickson's naturalistic and utilization approached directly engages reframing, which is an important mark of his work. Erickson developed an extraordinary sensitivity and observant capacity to know what was available to be utilized. This volume prepares the reader for developing their own observant sensitivity.

Vol 15: Mind Body Communication in Hypnosis provides an understanding of the connections between mind, brain and body and how they can affect each other. Erickson is the classic wounded healer and truly understands how the mind can generate healing throughout our biology. The very recent psychosocial genomic research by Cozzolino et al., is a fascinating investigation of the mind to body healing process through the changes to gene expression during the therapeutic hynosis technique developed by Ernest Rossi, the Mind-Body Healing Experience.

Vol 16: Creative Choice in Hypnosis is probably my favourite volume because it deals with the creative interplay within Ericksonian therapeutic hypnosis. Understanding how to be aware, responsible and yet improvising and non-directive is the gift of this wonderful volume. An insightful interview with Ernest Rossi from 1989, following Erickson's death is a both informative and touching recall of Milton Erickson and the work they did together.

Vol 17: The Wit and Wisdom of Milton H. Erickson is not yet completed, but I consider this to be the perfect conclusion. Erickson was well known for his wit and wisdom and the retelling of Ericksonian stories is always a joyful and valuable experience.

There is little doubt that this an excellent history of Milton H Erickson, his research and his practice, but this reader found it to be more. It is, for me, a personal journey. Not only am I informed, but I also experienced the wonder, fascination and tremendousness that, as Rossi explains, are the necessary elements to facilitate neural plasticity and activate gene expression and protein synthesis. This is how we generate personal growth and development. Jeffrey Zeig says (Vol 1, p xi) "He was consistently working, consistently being Milton H. Erickson, which entailed having the most profound experience he could with whomever he was sitting with." This, I believe, continues in these wonderful volumes.

Richard Hill - MBMSc, MEd, MA, DPC

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MIND-BODY TRANSFORMATIONS THERAPY (MBT-T)
A SINGLE CASE STUDY OF TRAUMA AND REHABILITATION:
THE PSYCHOSOCIAL AND CULTURAL EPIGENOMIC THEORY, RESEARCH AND PRACTICE OF THE NEW NEUROSCIENCE OF PSYCHOTHERAPY AND TRANSLATIONAL MEDICINE

Jane Mortimer, Kathryn Rossi & Ernest Rossi

ABSTRACT

This single case study of trauma and rehabilitation explores a new evidence-based psychotherapeutic protocol of Mind-Body Transformations Therapy (MBT-T). The psychological theory, research and practice of MBT-T is reviewed and illustrated with the transcript of a single 90 minute session of psychotherapy with a 59 year old professional woman who experienced a post-traumatic stress disorder (PTSD) initiated during her adolescence. Replication of this study with large clinical populations and controls is now required to confirm how the MBT-T protocol could supplement the traditional cognitive-behavioral dynamics of psychotherapy for ameliorating stress-related disorders with the neuroscience of the 4-stage creative cycle. We first demonstrate an emerging psychosocial theory for the rapid facilitation of therapeutic RNA/DNA mind-body transformations. We then generalize this demonstration with the MBT-T Self-Reporting Response Form for collecting group data for documenting a new psychosocial epigenomics protocol for applications to translational medicine in our supplementary materials.

INTRODUCTION

This single case study of the rehabilitation of a post-traumatic stress disorder illustrates the psychological theory, research and practice of a new psychosocial and cultural approach to the epigenomics of psychotherapy and rehabilitation suitable for large groups. Epigenomics is a scientific approach for exploring the interaction of nature and nurture: how genes interact with the environment to modulate behavior, cognition and consciousness (Bell & Robertson, 2011; Robinson, Fernald & clayton, 2008; Feinberg, 2007). Recent research in neuroscience, clinical and social psychology documents how complex psychosocial and cultural epigenomic mechanisms modulate gene expression without altering the DNA code (cole et al, 2005, 2007, 2009, 2010, & 2011). The Mind-Body Transformations Therapy (MBT-T) protocol focuses on a special class of epigenomic genes that are often described as "activity or experience-dependent genes," which can be turned on (activated) or off (suppressed) by signals from the physical and psychosocial environment that may be appropriate applications to translational medicine (Rossi, 1986, 1993, 2002, 2004, 2007, 2012; Rossi & Rossi, 2013; lloyd & Rossi, 1992, 2008).

SOURCES OF THE PSYCHOSOCIAL THEORY, RESEARCH AND PRACTICE OF MIND-BODY TRANSFORMATIONS THERAPY (MBT-T)

The theory, research and practice of Mind-Body Transformations Therapy (MBT-T) originated from the seminal studies of Milton H Erickson's therapeutic hypnosis and medical rehabilitation by Ernest Rossi in the 1970s (Rossi, Erickson-Klein & Rossi, 2008-2014). in his neuroscience update of Erickson's innovative cognitive-behavioural approaches to psychotherapy Rossi (Rossi, 2012, 2013; Rossi & Rossi, 2013) outlined how modern RNA/DNA microarray technology makes it possible to measure the expression levels of many thousands of genes simultaneously. This evidence-based research in psychosocial epigenomics has become a new standard in personalized and translational medicine (Eisen et al, 1998).

current research documents the use of RNA/DNA microarrays for assessing psychotherapeutic responses via a variety of top-down therapeutic protocols that were originally developed by many diverse cultural, historical and spiritual traditions of mind-body healing. These include the relaxation response (Dusek et al, 2008), therapeutic hypnosis (lichtenberg et al, 2000, 2004; Rossi, 2012; Rossi & Rossi, 2013), meditation (creswell et al, 2012) social regulation of gene expression (cole, 2009, cole et al, 2005, 2007, 20100, 2011), emotional freedom techniques (church et al, 2012; Yount et al, 2013), the therapeutic placebo (Sliwinski & Elkins, 2013) and yoga (lavretsky et al, 2013) to facilitate optimal states of human consciousness as well as the amelioration of stress related dysfunctions (Unternaehrer et al, 2012).

This single case study explores the hypothesis that such top-down therapeutic protocols epitomized by the Mind-Body Transformations Therapy (MBT-T) protocol could become the foundation of a more general theory of mind-body communication, meditation, psychotherapy and rehabilitation (Rossi, 2002, Rossi et al, 2008, Atkinson et al, 2010). A full description of the administration and scoring of the new top-down MBT-T protocol (originally named the "creative Psychosocial Genomic Healing Experience, cPGHE) is freely available (Rossi, 2012).

confirmation of the results of this single case study could update the cognitive-behavioral efficacy of evidence-based translational medicine and psychotherapy recommended as a standard of clinical excellence by insel (2009, 2010, & 2012), director of National institute of Mental Health.

In this paper we will first demonstrate the MBT-T with an individual and then outline how the MBT-T protocol can be used with groups.

A SINGLE 90-120 MINUTE MBT-T DEMONSTRATION OF PTSD REHA-BILITATION WITH ONE SUBJECT

Mind-body communication and transformations utilizes the normal circadian and 90-120 minute Basic Rest-Activity Cyclic (BRAC). Researchers are currently using time-series gene expres- sion data to facilitate how mind-body communication could facilitate health and recovery from trauma and stress related problems (Atkinson, et al, 2010) Bar-Joseph et al, 2012; Lloyd & Rossi, 1992, 2008, Rossi, et al, 2008; Qian et al, 2013). This single 90-120 minute demonstration of MBT-T explores and illustrates a variety of interesting and insightful approaches for modeling Mind-Body Transformations Therapy on all levels from mind to gene with the 4-stage creative cycle.

STAGE 1 OF THE CREATIVE CYCLE:

A FOCUSING OF ATTENTION AND POSITIVE EXPECTANCY WITH HAND BALANCING.

Rossi: May I demonstrate a gentle balance with your hand to help you learn how to deal with your own issues in your own way?
[Turns toward the audience] Milton H Erickson, MD would reach out with his hand and just lightly touch the wrist (Ernest Rossi touches underside of Charity's forearm and wrist. I then move my hand gently upward and across Charity's forearm to her wrist as a lift it. But my touch is so light that the client gets the tactil cues to lift her hand herself (See Fig.1).

Rossi: That's right, yes! Allowing that ... I am not grabbing her hand roughly but moving my hand very gently ... and I am not telling you to put it down.
[Turns toward the audience] And so you see Charity's hand re- mains suspended in midair. This is what Erickson called a "thera- peutic catalepsy," which is a position of balanced muscle tonicity and receptivity that is comfortable for the client. Erickson believed that this kind of balanced muscle tonicity led to what the modern neuroscience calls focused attention and a positive expectancy that something good is going to happen. It is the novelty of the surprising touch that activates the psychosocial epigenomic dynamics of the "novelty-numinosum-neurogenesis effect" (NNNE), to optimize the 4-stage creative cycle and prob- lem solving (Rossi, 2002) sumarized in Table 1.

The patient's private thoughts: Wow, I would like to open my mind but my arm does not feel comfortable so I will move my shoulder back a bit to be supported by the chair. It is aparant- ly important to be comfortable and balanced in this theory.

By facilitating this gentle hand balancing Rossi subtly focuses the client inward to experience focused attention with posi- tive expectations. This is what Erickson would call "shifting the bur- den of effective responsibility of psychotherapy onto the subject" (Erickson, 1964, in Rossi, Erickson-Klein Rossi, 2008 Vol. 3, pgs. 67-71).

The typical client begins with the attitude, "Oh, Doctor, please help me." Erickson's initial response was to immediately shift the burden of creative inner work with focused attention and posi- tive therapeutic expectation from the doctor to the patient so that she becomes active and receptive in expect- ing that some- thing good will happen with her new attitude of self-help!

This therapeutic self-help attitude is an important but little recognized psychosocial epigenomic secret of Milton H Er- ickson's therapeutic hypnosis. People liked to believe that Er- ickson was some sort of master hypnotic manipulator. While this desig- nation may have some superficial appearence truth, Erickson actually taught simple effective techniques for activating the patients own inner focus and creative process for inner problem solving.

Rossi: [Ernest pauses for 20 seconds looking at Charity. Such creative pauses allow her to focus on private experiences with positive expectation.]

STAGE 2 OF THE CREATIVE CYCLE:

INTRODUCING THE NNNE AND THE PSYCHO- SOCIAL EPIG-ENOMICS OF THE MBT-T.

Rossi: [Turns towards the audience] Your introducing the sub- ject to an experience of novelty, which for Erickson often meant experiences of unusual but comforting hand touch. This hand balancing initially focused the subjects attention with positive expectancy which Erickson would then pro- ceed to help the subject explore some interesting questions about themselves, their past, etc. He would start to build on positive expectations and focused attention in appropriate and unique ways for every individual. He would ask people to explore some of their earliest memories. What are some of the earliest things they learned? How did they learn how to read? Could they see the first book they read? Could they remember back even further to when they learned how to walk? This early memory review facilitates activation of the NNNE in turning on the molecular level of experience-depen- dent epigenomic expression for rehabilitation and problem solving.

The patient's private thoughts: Yes, I can remember looking at pictures and learning to read letters one by one, and I do have memories of when I learned to walk!

Rossi: So you continue, Charity, with your earliest memories of writing. I don't know if you can actually experience ... (cre- ative pause) what some of your earliest focusing was? What were some of your earliest words when you were learning how to write? What did they looked like? I don't know if you can go back even further learning how to ... Yes, what is the earli- est memory of your learning how to do something? Can you remember talking to yourself to help you learn?
The patient's private thoughts: Yes, I can remember the book 'The Little Bear' with its sweet watercolor images, black spine

binding and blue mottled paper cover with a small line drawing of the Little Bear.

Rossi: That little girl ... What did they call you as a little girl?
The patient's private thoughts: "Child," they called me" Child!"

Rossi: I don't know if you can remember ... Yes ... What was the first birthday you can remember? ... How old were you going to be? ... Do you remember what were the first gifts you received? ... And so continuing privately within yourself now, Charity, go- ing from one birthday to the next.

The patient's private thoughts: Age 4 I received my doll that I named "Nancy" for my birthday. Nancy had brown hair and a pretty blue brocade dress my grandmother made for her. My grandmother made a trunk full of doll clothes for each of my many dolls throughout my childhood.

Rossi: A year older ... Growing from one birthday to the next ... gradually until you are as old as the bigger kids

The patient's private thoughts: At age 5 I am all grown up and full formed. I see my face, my dress, my birthday cake with pretty pink roses and my new doll Debby. Debby was my favorite doll of all time and I still have her in my closet today.

Rossi: I don't know if you can remember age 7, 8, 9?
The patient's private thoughts: I see my face clearly at each age and remember my birthday parties.

Rossi: And the Yes, becoming a ... teen? Age 11, 12, 13 ...

The patient's private thoughts: Age 11 I joined the orchid society. Age 12 was when the first cute surfer boy asked my father for permission to date me – which my father declined. Age 13 when traumas began after my father died unexpectedly and my mother remarried.

Rossi: Yes ... some of the best experiences ... some of the ... not so great experiences (Rossi quietly and warmly laughs) ...

The patient's private thoughts: This is when the hell begins!!! [Explamation points as she begins to access the adolescent sources of her PTSD.] How soon can we get this over with? Can't I just stay with happy memories? I just need to have the courage to stay fully with this age ascending since I know it is going to be time limited and I won't have to stay here long in therapy.

Rossi: Some of the high hopes ... and some of the worst of those earliest ... What you are going to be when you grow up?

The patient's private thoughts: I'm going to be a psychologist and help people starting with me. That's what I am going to do, and did. I began to read books trying to learn ho beginning with 'I'm OK, You're OK".

Rossi: And now moving into 15, 16, 17 Wow! ... When did you know you were ... going to college?

The patient's private thoughts: The lost years of the worst of my trauma, abandonment, loneliness and dissociation. I gradu- ated from High School early at age 17, took the weekend off and then began college just to get a head start on

living my life as I dreamed it could be.

Rossi: Yes. What was that young lady ... What were the thoughts of herself and her future?

The patient's private thoughts: I am determined to create an independent life of my own where I don't have to depend on anyone to take care of me. I do not want to be stuck on the whims of others.

Rossi: How was it learning new things? ... Really focusing in on all the transitions between adolescence, becoming a young adult ... going to college.

The patient's private thoughts: It is always hard learning new things. Outwardly I was confident but inwardly very insecure.

Rossi: I don't know if you remember the first day... the first time you entered your dormitory or place where you lived in going to college. I don't even know if you lived away from home. [Turns toward the audience] This 'I don't know' technique is a therapeutic inquiry where the client takes responsibility for themselves, as I cannot possibly know everything. i.e. notice that the therapist really does not need to know everything! However, the therapist does need to know how to facilitate the patient's private thoughts that activate the NNNE to optimize the epig- enomics of self-help in translational medicine.

The patient's private thoughts: My first day of college in the win- ter was very rainy and cold. My small college had old military Quonset huts for buildings with wood stoves for heat and fan- tastic teachers. I guess I never told anyone about my living situ- ations in college. I lived on Atoll Street for a little while sharing a room with my brother who worked at night when I slept, and he slept during the day while I was at school. Fortunately that only lasted a few months before I had my own room to myself.

Rossi: What were some of those first collage classes? ... Just watching yourself going to them ... one semester to the next ... some of the really surprising things ... some, I suppose, disap- pointments? ...

The patient's private thoughts: I loved my classes of Shakespeare, American History, Physiology and survey Psychology classes. One English teacher was so condescending and terrible. He marked my papers with so much red ink that I couldn't see my words, while my Shakespeare teacher guaranteed me a "C" if I would simply write my essays. He wanted to help correct the negativity from the first English teacher. My physiology teacher did the same as I wanted to drop the class since I thought I was too stupid to pass. He assured me that I was the only non-nurse in the class and as such I couldn't possibly know, or learn as
fast as they could. Both professors had a profound effect on me through their kindness and eyes to see that I really could succeed.

Rossi: And then moving on to the miracle of all miracles, gradu- ating ... but still not finished. The part of you that wanted to go on to graduate school ... and watching that whole process of graduate school ... working on your doctorate ... frustrations, breakthroughs ...

The patient's private thoughts: My Bachelors Degree in psy-chol- ogy was useless because I could not afford to take a job in my field since the pay was too low.

Rossi: And finally graduation. And was that? ... You would be a graduate, a professional and learning to become who you are today.

The patient's private thoughts: I got a Masters of Arts Degree and still not afford to take a pay cut to work in in the field of psychology. I had to go on to earn a doctorate. There were too many traumas along the way to think about all of them. Some of my professors wanted me to fail while some profes-sors put me on a pedestal just so they could knock me down later. I finally graduated with top honors by not listening to the ones who wanted me to fail, nor to the one's who over-idolized me. Even though I broke my neck nearly becoming paralyzed, I persevered because I wanted to lead a life where I could take care of myself as I promised myself in my teen years.

STAGE 3 OF THE CREATIVE CYCLE:

FACILITATING THE AHA! THERAPEUTIC EX- PERIENCE WITH THE PRIVATE INNER SELF OBSERVER.

Rossi: I wonder if you can see the whole thing in perspective? Looking at that young child first learning ... all the way to the young adult? Yes, that private inner self observer ... knowing the simple truth that only you could possibly know. How will you use your inner observer to help yourself now and in the future?

The patient's private thoughts: Yes. I am the same person throughout the years even though I broke my neck and success- fully rehabilited myself. Twenty-five years later had another serious traumatic head injury that I also successfully rehabbed. I am proud of my recoveries. Come to think of it – I have always known how to help myself.

Rossi: And continuing on to today in your growing maturity ... That's right, seeing and feeling your whole self, that whole jour- ney from childhood to today ... That is all of it is one story. The story of ... Yes, what would you call the story of ...

STAGE 4 OF THE CREATIVE CYCLE:

FACILITATING THE SELF-HELP EPIGENOM- ICS OF TRANSLA-TIONAL MEDICINE VIA THE MBT-T.

Rossi: So continue focusing in your own mind privately ... get-ting this marvelous perspective ... opening your mind. This incredible journey you have been on.
[Turns toward the audience] By allowing people to have their own private experiences, without the need to confess every-thing the therapist bypasses most of the persons so-called "inner re- sistence" so their natural 4-stage creative cycle can proceed with optimal freedom and self-help!

The patient's private thoughts: There truly is a whole continu-ity to who I was as a young child and who I am today.

Rossi: And when you know that ... in a couple of minutes you can come back to the room fully alert, conscious, and ready to begin a ... deeper exploration of how you can work with yourself ... effectively ... optimizing your learning, healing and well-being in the wonderful adult you are now. When you know you can do that let's see whether your eyes will open first, or will you stretch first? [These are the realistic behav-ioral inevitabilities that are signaling the end of the persons creative inner work.] Coming completely alert for the new process of inner optimizing creative ... mind and well-being.

Rossi: Let's see how you are. Do you feel ready, for example Char- ity, to move on, or is there something else that you want to share that is appropriate to share with the audience at this time?

Charity: That was really beautiful (therapeutic tears) about the Continuity of My Life. I've always know who I am from the earli- est days. I can clearly remember my 4th birthday. By the time
I was age five I deeply knew who I was. I distinctly remember thinking that I was all grown up and the age of 5, and do you know what? I was. There were a lot of things I didn't know yet. I was still a kid and needed to be taken care of. Even though I didn't have a full adult mind, I knew I was fully grown and fully formed by the time I was 5. I was a little lady at that point and I needed private time. I needed a lot of private time at the age of 5.

Rossi: Really?

Charity: Yes I did. I knew at the age of 5 what I know to be important today:
•You have to be smart and think things through.
•You have to let people come to their own conclusions. You can- not tell people what their conclusions are, and certainly nobody can tell me what my conclusions are!
•You cannot tell me what to think or believe just as I cannot tell you what to think and I cannot tell you what to believe. I really understood that all at the age of 5.

Rossi: Thank you. That is a beautiful summary of your personal path to self-help and recovery.

ONE WEEK FOLLOW-UP

A week Later Charity reports another profound MBT-T group ex- perience wherein she found her neck gently elongating in a con- tinuing healing and freedom from her head and neck injuries.
Charity: It was really weird that I could not get comfortable dur- ing this week on account of neck pain. I stood up, sat down, lay down and tried to exercise to see if the pain in my neck would go away.
Finally, I realized that this was a continuation of last weeks' therapeutic experience.
I simply tuned in and allowed memories of breaking my neck and other concussions to come up.
Rapidly, a kalidiscope of memories ping-ponged in my brain and then my neck simply let go!
I was so surpirsed to have new neck length and freedom from pain and freedom of movement.
If you have an open mind you never know how far you can go with self healing!

FACILITATING THE 4-STAGE CREATIVE CYCLE IN THE MBT-T:

THE ROLES OF THE THERAPIST AND THE PATIENT

This case study reflects an example of Mind-Body Transformations Therapy (MBT-T) (formerly described as the Creative Psychosocial Genomic Healing Experience). This therapy consists of utilizing the our natural 4-stage Creative Cycle in self-help.

STAGE ONE: SELF EXPLORATION

Stage 1 involves preparation and self exploration. The role of the therapist in this stage is to allow the client to use arousal and stress to motivate the client toward problem solving and healing. It is not the role of the therapist at this stage to alleviate emo- tional distress. Arousal is an inner stage of therapy that triggers a creative process of problem solving as illustrated in figure 2.

STAGE TWO: THE DARK NIGHT OF THE SOUL: PRIVACY & IMPLICIT PROCESSING HEURISTICS

Stage 2 often involves the patient in inner conflicts and feeling stuck, which may elicit negative memories and abreactive emo- tions.
This stage can be accompanied by crying, frowning, and feelings of inadequacy, stress and depression.
The most important role of the therapist during this stage is to support the client's private inner work. Facilitating private inner work more often than not involves replaying painful past memo- ries that are the source of the problem.
The client is supported by the therapist's indirect permissive sug- gestions (also known as implicit processing heuristics). For example, the therapist may ask in Stage 2:
Will it be okay to allow yourself to continue replaying that privately for awhile ... difficult though it may be, so that you can learn what you need for healing [problem solving, etc?... [See top of fig. 2].

Can you let yourself continue to experience that for another mo- ment or two in a private manner—only long enough to experi- ence what it leads to next?,
And will it be okay to replay that trauma again privately in a way that you would really like to experience it? ...
This is also a time in which the therapist may need to assist the client to reframe negativity and confusion. Reframing confusion as a creative transition to stage 3 Aha! can be very therapeutic. For example, the therapist may ask the client, Have you ever experienced confusion before learning something new?
The most common error that therapists make during this stage is to offer advice that interrupts the client's private inner work and may stop her from working through the negative emotional arousal. The therapist can shift the primary burden of responsi- bility of effective therapy onto the client by using simple implicit processing heuristics such as, Knowing you can continue receiving whatever comes up all by itself and saying a few words about it whenever you need to, but only what I need to hear to help you further.
These and many other novel and innovative techniques for facilitating stage two of the 4-stage creative cycle with the MBT-T helps the patients bypass their so-called "inner Freudian resistances" (Rossi, 1993, 2002, 2007, 2012) and focus on their positive inner resources to greatly shorten the total number of sessions (typically 2 to 10) required for effective brief psycho- therapy.
•Respecting patient's privacy
•Patient's inner work
•Sleep-Dreams-Early morning Thoughts
•Implicit processing heuristics
•Self-observer, self-healer, positive self-talk •Novelty-numino-sum-neurogenesis-effect (NNNE) •Positive continuity of life review
•Extended 90-120 minute sessions (BRAC)

STAGE 3: THE AHA! CREATIVE MOMENT

Facilitating Stage 3 of the 4-stage creative cycle, the Aha! Mo- ments, is the essence and high point of the MBT-T. Stage 3 usually surfaces as a result from the private inner work of replaying the origins of the problem in Stage 2. There may be a slight smile of surprise with the emergence of stage 3 and the head may nod positively slowly with minimal movement. In Stage 3 new solu- tions or insights are created along with positive self change. It is not unusual for clients to shift slowly back and forth between stages 2 and 3. Clients often need help during this transition to recognize the value of their creative insights, especially if such insights were not valued or supported during childhood.

STAGE 4: GIVING YOURSELF YOUR OWN BEHAVIORAL PRESCRIPTION FOR EVERYDAY LIFE

Stage 4 leads to verification of positive experiences and be- hav- ioral prescriptions that come from the client themsleves. It is a fundamental part of MBT-T to take the insights gained in Stage 3 and apply them to make changes in real life. The client can be gently supported to do this by asking questions such as: How can this experience change your life?
How will you use this to make changes in your life?
What will you actually do in your life that is different this week?

In this way, symptoms are reframed as signals and problems can be reframed into opportunities through accessing inner resources.

MBT-T emphasizes the use of creative mind-body cycles that occur approximately every 90 to 120 minutes (also known as ultradian rhythms (Lloyd & Rossi, 1992, 2008).

The client is encouraged to explore such mind-body cycles in everyday life, taking a 20 minute break every 90 to 120 minutes and tuning into themselves in a sensitive and compassionate way, keeping a written record of experiences of anything new that comes up during this resting phase and his or her early morning thoughts.

This can provide useful hints for therapeutic work in the next session.

Clients can be told that they will go through 12 creative work cycles each day, which amounts to about 84 possibilities each week, to make positive changes in their lives and solve their problems. With this opportunity clients may they learn to find the resources within themselves.

SUMMARY

The theory, research and practice of Mind-Body Transformations Therapy (MBT-T) is a new psychosocial and cultural epigenomic method for facilitating recovery from post-traumatic stress disorder (PTSD) that is suitable for enhancing the new neuroscience of psychotherapy and translational medicine. A single session clinical demonstration with a with a 59 year old woman illustrates the details of a variety of novel neuroscientific epigenomic mechanisms that are hypothesized to facilitate the natural 4-stage creative cycle of self-help on many levels from private experiencing to the molecular dynamics of experience-dependent gene expression. Supplementary materials illustrate how this single session of MBT-T can be adapted for applications to large groups engaged in self-help programs for recovery from a variety of stress related disorders. This paper introduces a novel top-down psychosocial and cultural epigenomic approach to supplement the traditional bottoms-up molecular-genomic approach to translational medicine. We now recommend further research to assess the degree to which this top-down approach enhances the evidence-based efficacy of the traditional molecular-genomic applications of rehabilitation and translational medicine.

SUPPLEMENTAL MATERIAL

WORKSHOP 28 AS A DEMONSTRATION OF THE NNNE.

This handout was used in our workshop with large groups (~200 therapists) at the December 2013 Evolution of Psychotherapy Con- ference presented by the Milton H Erickson Foundation. This handout, or appropriate parts of it, are used to begin facilitating the Novelty-Numinosum, Neurogenesis Effect (NNNE) of the entire audience. The MBT-T was then administered to everyone. For profes- sionals in mental health the novelty of much of the new information in the PowerPoint presentation of this handout tended to evoke the numinosum as a motivating factor that sometimes evoked the psychosocial activation of epigenomics of experience-dependent gene expression and neurogenesis to facilitate the molecular genomics of the self-help appropriate for psychotherapy and transla- tional medicine. This PowerPoint tends to evoke the NNNE just as the hand balancing therapeutic approach, which was used in the single session MBT-T experience with the 59 year old woman in the main section of this paper.

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THE PSYCHOBIOLOGICAL EFFECT OF THE ONLINE SELF-HELP PROGRAM '15MINUTES4ME.COM'

Paul Koeck

SUMMARY

Background
Modern patients search for solutions on Google. This creates a new segment of patients who are willing to do professional self-help therapy without tradition psychotherapy.

Program
Patients with stress, anxiety, depression or burn-out, followed the online auto therapy self-help computer program '15Minutes4Me.com(version 1.0)', during 15 minutes per day. The program helped them to (re)discover how to (re)build the meaningful life they search for, and how to develop new 'desired habits' to (re)create their desired meaningful life without suffering, stress, anxiety or depression.

Methods
In a retrospective study on 1056 participants following the '15Minutes4Me.com(version 1.0)' auto therapy self-help program, each 7 days the DASS-21 test was taken to measure evolution of their stress, anxiety and depression.

Findings
We found significant improvement with a strong correlation (p= •000, r=•59) after 3 weeks of daily participation. The average total DASS-21 score improves with 50% within 21 days, while the average participant reaches normal values within 28 days of participation.
This indicates this program is a valid tool for further exploration to research, with microarray DNA techniques, if alteration of gene expression during this program gives a potential insight in the potential healing mechanism of psychosomatic illnesses.

INTRODUCTION

Background
Modern patients tend to search for solutions on Google before seeing a physician or psychotherapist. Some will even never see a health care professional because they fear talking to someone about their problems. However, some of them are willing to try an online solution in the form of a digitalized self-help auto therapy program. In order to address their needs, the author started a research project to study how internet self-help therapy can help them modify their psychological and biological stress-related condition or disease.

Rationale
This study is part of a larger research project aiming at studying the effect of a self-help auto therapy program on psychological, biological and psychogenetic parameters in patients who choose for internet self-help with an internet software program.

In this study we want to present the first results of a retrospective study on 1056 participants following the '15Minutes4Me.com(version 1.0)' program between 1st of April 2011 and the 20th of June 2014 in Dutch language. The Dutch translation of the name of this program is 'MijnKwartier.be(1.0)' for the participants in Belgium and 'MijnKwartier.nl(1.0)'for participants in the Netherlands.

This retrospective study is part of a preparatory effort for future research on the question if online self-help psychotherapy does turn on the expression of certain genes as is found in the research project by Cozzolino et al. on a hypnotherapy intervention designed by Ernest Rossi. They found that in 200 genes expression is modified by one hypnotherapeutic psychotherapy session, some one hour after therapy, others 24 hours after the therapy session. We would like to study in the future if online self-help therapy does alter gene expression within participants after succeful self-help therapy. To prepare for this future research, we try to analyze in this study if and how fast patient normalize their psychological stress levels as measured with a simple questionnaire. We assume that this is important in order to investigate if and how certain psychosomatic conditions can be cured, on top of the actual psychological applications of this program.

In this retrospective study results were evaluated with the use of the DASS-21 scale developed by Lovibond and Lovibond at the University of Queensland in Australia. Participants took this test electronically every 7 sessions. The program offers one session of about 15 minutes per participation day. Participants follow the '15Minutes4Me.com(1.0)' program from their home computer, tablet or smartphone and are recommended to do one session per day, with the freedom to skip from time to time a day, either when they have no internet access or when they need a day-off from doing auto therapy.

The results of this study will be used to improve the quality of the development of '15Minutes4Me.com(version 2.0)', the next version of the program and to design the protocol for the future psycho genomic study. We assume that 'normalization' of DASS-21 scores could indicate the optimal timing to measure eventual modification of gene expression with the microarray DNA technique.

Research Questions
The aim of this research is to study following questions:

1. Do participants make a significant progress during the participation at the self-help program '15Minutes4Me.com(1.0)'?

2. Do participants reach 'normal' levels of stress, depression or anxiety during their participation at the self-help program '15Minutes4Me.com(1.0)'? and when?

METHOD

Study Design
This study is a retrospective analysis of the DASS-21 scores of participants who followed the self-help auto therapy program '15Minutes4Me.com(1.0)' between April 1st 2011 and June 20th 2014 during at least 8 sessions. Participants found the program via Google, a friend or their physician and paid 55€ for one calendar month and were recommended to attend one online self-help session of about 15 minutes every day. It was technically impossible to do more than one session per 24 hours. After one calendar month, they had to make a proactive decision to pay another 55€ for one additional calendar month or 99 € for 2 additional calendar months of access to the program. They received a certificate of attendance if they participated during more than 75% of their 30 or 31 calendar days. This certificate can be used to request financial reimbursement by either their employer or their medical health insurance. They are free to choose how many days they attend the program, equal to what happens in many settings of traditional psychotherapy.
Every seven sessions or participation days, they took the DASS-21 questionnaire as a normal element of their self-help program, since those results are used as part of the software program.
On the 20th of June 2014, the data were exported from the program for analysis with MS Excel or SPSS. The data included scores on the three subscales of the DASS-21 (depression, stress and anxiety) for each participant. To be included in this study, participants needed to complete 8 daily sessions or more between the first of April 2011 and the 20th of June

2014. 1056 particpants followed 8 or more daily sessions. All participants signed an informed consent as part of their registration procedure.

Self-help Philosophy and Interventions
The main philosophy of the '15Minutes4Me.com(1.0)' program is to help participants help themselves utilize their own resources and the available or accessible resources in their social context in order to make better choices to live the meaningful lives they want to live.
Milton H. Erickson, MD and Ernest Rossi, PhD define the 'utilization' approach as follows: 'facilitate the utilization of abilities and potentials that already exist within a person but that remain unused or underdeveloped because of a lack of training or understanding.'

Viktor Frankl, MD developed the idea that mental health is connected to the ability to live a meaningful life. Luc Isebaert, MD expands on this concept stating that the goal of therapy is "to help clients to live the (meaningful) life they desire to live, and to choose freely what habits the client wants to develop." The Greek philosopher Artistotle already introduced the concept of 'habit' in this book 'Rethoric': "Acts are done from habit which men do because they have often done them before".

The '15Minutes4Me.com(1.0)' program helps participants become aware of what meaningful life they want to live and what habits they want to develop in order to be able to live that meaningful life. In order to develop those 'desired habits' the program asks participants self-reflective questions to help them remember and rediscover what useful abilities and other resources they already have in their life. Reactivating their memories for previous resources, solutions - and abilities helps them (re)develop those underdeveloped or unused potentials. In order to transform this newly rediscovered abilities into 'desired' habits, the '15Minutes4Me.com(1.0)' program will invite participants to reactivate this reflection during about 15 minutes per day, until those solutions become habits. This program helps participants to choose develop the required habits that will contribute to help them to live the meaningful lives they desire to live.

Besides stimulating reflection about existing abilities and potentials, the '15Minutes4Me.com(1.0)' program offers specific psycho education adapted to the individual needs of each participant, in the form of video, animations or words. Further scaling questions , progression graphs and other interventions to summarize and visualize resources, abilities and potentials are used in this self-help program. Virtual buddies can be invited electronically to stimulate social support and access natural resources from the participant's social network and context.

Every week participants received a report with evolution graphs for their General Physician since the program does not pretend to replace their physician. The '15Minutes4Me.com(1.0)' program wants to be a useful additional tool for physicians to choose from when treating a patient with stress-related disease. It is a new class of therapeutic tools, just as psychoanalysis, brief therapies, meditation, hypnotherapy or psychopharmacologic medications can be a valuable therapeutic tool. Classes of therapeutic tools do not always replace each other but often supplement each other to assure

a better treatment for the patient that fits to his life style, individual choices and personality.

Outcomes

The DASS-21 (Depression, Anxiety and Stress Scale) is a dimensional scale that measures depression, stress and anxiety. It has a high internal consistency and can be used to measure change over time. The DASS-21 consists of 21 items, 7 measuring depression, 7 measuring anxiety and 7 measuring stress. The self-report scale uses a Likert-scale from 0, meaning never or practically never, to 4, meaning most of the time or all the time . The DASS-21 was compared with the BDI (r =0.74 with depression subscale) and BAI (r =0•81 with anxiety subscale) and showed high correlations. The depression subscale has a reliability index of 0.91, the anxiety subscale an index of 0.84 and the stress subscale an index of 0.90. In this study, the Dutch version of the DASS-21 was used, with reliability indexes of 0•91 (depression), 0•86 (anxiety) and 0•85 (stress) . The validity of the Dutch version was high (r =0•72 with the BDI, r =0•77 with the BAI). The related variability was significant (p =0•001).

To address potential sources of bias, there were a few meaures taken in this study. First, to standardize the therapy, a computer program was used. This way, all the participants had the same type of interventions, questions… The DASS-21 was programmed to be taken at the daily session N° 1,8,15 and 22 by each participant. Second, the DASS-21 had a high reliability and validity.

RESULTS

Question 1: Do participants make a significant progress during their participation at the self-help program '15Minutes4Me.com'?

Total DASS Scores

Figure 1 shows the evolution of the DASS-21 scores of participants during the '15Minutes4Me.com(1.0)'self-help program in function of the amount of (daily) sessions attended. The first row displays the amount of participants at that specific point in time. Next the 75th percentile, average, mean and 25th percentile are displayed. The number of participants who are still participating and taking the DASS-21 test are displayed in a bar graph as 'count'

A repeated measures analysis with a Greenhouse-Geisser correction determined that the score on the subscales after 21 daily sessions differed statistically significantly and with a strong correlation (p= •000, r=•59).

This significance is visualized in the declining DASS-21 scores over time for the 75th percentile, average, mean and 25th percentile as seen in figure 1.

Figure 2 displays the relative decline in DASS-21 scores of each participant expressed in % of evolution from his initial score on the day he or she started the program. The average declines with over 50% within 21 sessions and the mean improves with 50% somewhere between the 15th and 22nd daily session.

DASS Sub Scores

Figure 4 displays the relative evolution participants make over their sessions, since their entry score at day one for each sub scale. On the sub scale stress, the average sub score decreases with 50% within 28 days. On the sub scale of anxiety this happens within 21 days and on the depression sub scale this happened within between 14 to 21 days.

Question 2: Do participants reach 'normal' levels of stress, depression or anxiety during their participation at the self-help program '15Minutes4Me.com(1.0)'?

Total DASS

The total DASS-21 score reaches – according to Lovibond & Lovibond - 'normal' levels once below the value 30, as indicated with the arrow on the graph. The average score normalizes between 22 and 29 sessions. The mean normalizes between the 15th and 22nd daily session, as one can see in figure 1.

DASS Sub Scores

In figure 3, one finds the evolution of average DASS-21 sub scores over time, expressed in the amount of daily sessions participated in the '15Minutes4Me.com(1.0)'self-help program. The first three rows in the table show the evolution of the sub scale for respectively stress, depression an anxiety. The last three rows, show the level below which the score becomes 'normal' on that specific subscale for respectively stress, depression and anxiety. In the legend, this is indicated with 'No Stress','No Depression' and 'No Anxiety'.

The average for the sub scale 'stress' reaches normal values within 21 daily sessions. For the sub scale 'depression' this happened within the 21th and 28th daily session. For Anxiety around the 36th daily session. The cross over points where the sub scale becomes lower than the threshold for normalization, are indicated with an arrow in figure 3.

Total DASS Scores

	1	8	15	22	29	36	43	50	57	64	71	78
Count	1056	1053	858	646	410	290	249	211	155	111	92	72
Percentile 75	78	56	48	44	38	36	34	34	32	34	28,5	30
Average	63,56	47,57	36,57	31,64	78,48	76,86	75,10	75,07	74,06	77,85	19,85	19,06
mean	64	40	34	28	26	24	20	22	18	18	16	15
Percentile 25	48	28	22	16	14	14	12	8	9	10	8	8

Relative Total DASS Evolution in %

	1	8	15	22	29	36	43	50	57	64	71	78
Count	1056	1053	858	646	410	290	249	211	155	111	92	72
Percentile 75	0%	28,21%	38,46%	43,59%	-51,28%	-53,85%	56,41%	56,41%	58,97%	56,41%	63,46%	-61,54%
Average	0%	-33,11%	-47,54%	-50,27%	-55,19%	-57,74%	-60,51%	-60,56%	-67,14%	-64,06%	-68,77%	-70,07%
mean	0%	-37,50%	-46,88%	-56,25%	-59,38%	-62,50%	-68,75%	-65,63%	-71,88%	-71,88%	-75,00%	-76,56%
Percentile 25	0%	-41,67%	-54,17%	-66,67%	-70,83%	-70,83%	-75,00%	-83,33%	-81,25%	-79,17%	-83,33%	-83,33%

DASS sub scores + crossing point to 'Normal'

	1	8	15	22	29	36	43	50	57	64	71	78
Stress	26,00	18,02	15,81	13,77	12,80	12,18	11,29	11,05	10,85	10,52	9,13	9,72
Depression	20,78	13,35	11,42	9,56	8,27	7,66	7,00	7,14	7,24	6,40	5,61	5,17
Anxiety	16,79	11,15	9,29	8,31	7,41	7,03	6,82	6,88	5,97	5,93	5,11	4,17
No Stress	14	14	14	14	14	14	14	14	14	14	14	14
No Depression	9	9	9	9	9	9	9	9	9	9	9	9
No anxiety	7	7	7	7	7	7	7	7	7	7	7	7

Relative Improvement of DASS sub scores

	1	8	15	22	29	36	43	50	57	64	71	78
Stress	0,00%	-30,68%	-39,19%	-47,02%	-50,75%	-53,15%	-56,59%	-57,49%	-58,26%	-59,53%	-64,88%	-62,60%
Depression	0,00%	-35,75%	-45,05%	-54,00%	-60,20%	-63,15%	-66,33%	-65,65%	-65,16%	-69,21%	-73,00%	-75,13%
Anxiety	0,00%	-33,60%	-44,63%	-50,52%	-55,86%	-58,13%	-59,38%	-59,00%	-64,41%	-64,69%	-69,57%	-75,18%

DISCUSSION

Context

The goal of this specific study is to prepare the field for further psychgenomic research in line with the findings by Prof. Mauro Cozzolino and Dr. Enest Rossi. They were studying the impact on gene expression of a single session hypnotherapeutic intervention.

Our intention is to study the impact on gene expression of participating in the online self-help program '15Minutes4Me.com(1.0)', until normal DASS-21 scores are reached by the participant whom before participation had pathologic scores on Stress, Anxiety , Depression or a combination of those. This is because we expect that the transition from pathological scores to normal ones might imply many changes in gene expression, and probably in different genes than what was observed with a single session hypnotherapy session. We hope to study in future research how the result of having helped patients make better choices (and develop corresponding habits) to live the meaningful lives they (un)consciously desire to live. The use of a computerized software self-help program allows to eliminate every form of human bias by the therapist or researcher.

Because we will want to plan adequately the blood sample timing, we decided to study in this article the evolution of participants, while trying to understand how they evolve over time and by when they reach normal values in their levels of stress, anxiety or depression. This should correspond to the moment where they have developed the required desired habits to live their own meaningful life.

Key Results

The average evolution of participants over time is significant with a strong correlation as measured over the first 3 weeks of participation in the '15Minutes4Me.com(1.0)' program.

Average total DASS scores improve with 50% within 21 days (Figure 2). The average total DASS score normalizes within less than 28 days, while the mean normalizes within 21 days (arrows in figure 1). The average individual DASS subscales normalize within 21days for Stress, within less than 29 days for Depression and within 36 days for anxiety (arrows on graph in figure 3). And in figure 4 one can see that the half-life time for the stress sub scale is 28 days, for depression less than 21 days and for anxiety 21 days.

Taking into account the significant improvement with strong correlation and the reasonable time span for the average participant to reach normal values on the total DASS scores - as well as on the individual sub scales-, this program seems to be well suited for future analysis of potential changes in gene expression of human leukocytes with microarray DNA techniques as described by Cozzolino et al.

They found a kind of domino effect where one hour after the hypnotherapy session 46 genes were altered in there expression, leading to a new cascade of changes 154 additional genes were altered in expression.
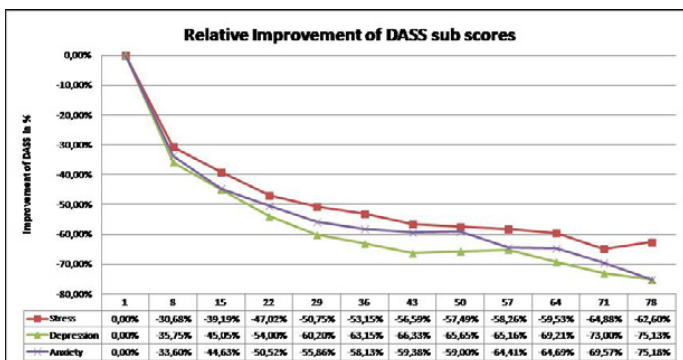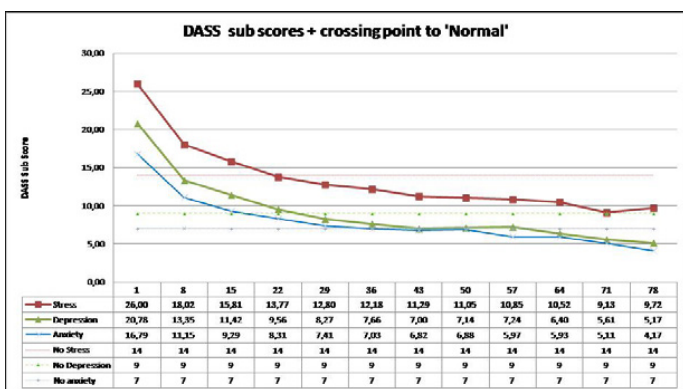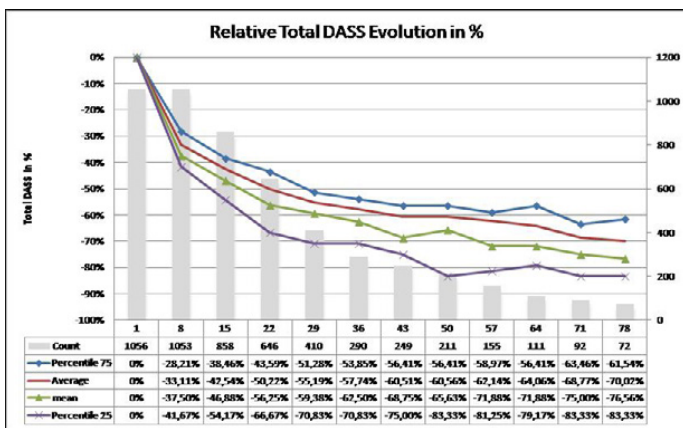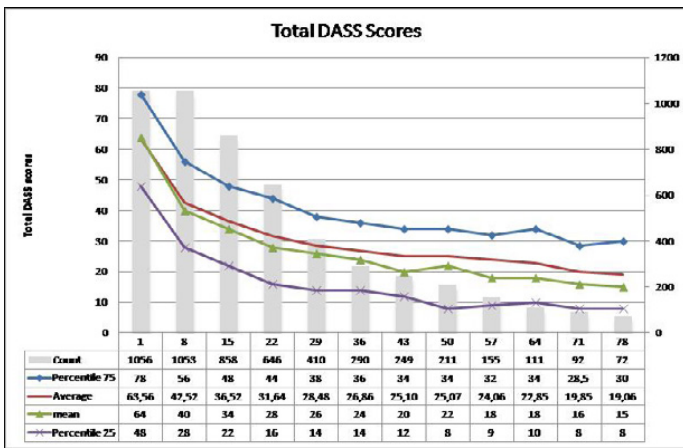
It seems – by analogy – reasonable to assume that a first set of altered gene expressions can be expected as soon as participants of the program enter the zone of normal DASS scores, potentially followed by a cascade of other gene expression patterns some weeks later when the newly acquired behavioral patterns are becoming solid habits. In other words: when the participant has learned to turn the daily choice for the newly developed 'desired habit' into a meta-habit by itself. It is probable that at that point other gene expression patterns will appear.

One could say that at a first level, the participant first develops a new habit, and then still has to continue reinforcing this pattern in order to learn to choose to make the daily choice for this desired habit a meta-habit in itself. That is the point where relapse under normal circumstances becomes less likely.

Importance

Why is this investigation important? Until now the scientific community knows that good psychotherapy has some effect on measured levels of stress, anxiety or depression. This has lead to further investigation of the mechanisms involved on a neuro-anatomical level, or in the sphere of hormones, such as the effect of increased cortisol levels in the development or cure of depression. Clinicians also observe that many psychosomatic diseases, or even somatic conditions like auto-immune diseases, Crohn disease, allergies and even cancers are influenced by stress related factors. If one would find specific genes being turned on or off after participation in an auto therapy program until durable normalization of stress scores, we might discover new pathways to understand the onset or offset of psychopathology versus healing of such conditions.

The first great benefit is that it could give hints to future researchers as well in the clinical psychological area as in the medical field to develop new therapeutic strategies and interventions.

Another benefit of such research will be that it helps clinicians to identify more accurately, which patients can potentially benefit from auto therapy or psychotherapy.

We are grateful that the research by Cozzolino et al opened new insights in how therapy as a process works on a psycho genomic level. We now hope to attract through this publication academic partnerships to participate in researching how the '15Minutes4Me.com(version 1.0)' program does impact gene expression for genes involved in the psychopathology of somatic as well as psychiatric diseases.

Limitations

Since this study is part of a larger research project, we did not research yet long term follow-up of patients after quitting the program, neither did we compare results to a control group. Both of those issues will be addressed in a following research project that is in preparation for the moment.

Another point is the 'openness' of the psychological contract with the participant. Just as in most psychotherapy settings, clinicians have no control how long patients will follow their

psychotherapy. The medical model of thinking assumes one should follow the patient until he is symptom free as an indication of 'cure'. Several psychological models, however, based on the 'utilization' approach, assume clinicians need to see the patient as long as the patient needs to learn how to utilize his resources. This has as a consequence that many patients quit the therapeutic relationship with the therapist, long before they are symptom-free. They quit as soon as they know how to proceed by themselves, as soon as they know how to self-heal themselves by leveraging on their self-healing resources, rediscovered during the therapeutic relationship. The same model is used in the design of the '15Minutes4Me.com(1.0)' program. Patients choose freely when to quit or discontinue the program. In a previous study on 545 patients, we analyzed when patients discontinue this program : 42% discontinues after reaching normal scores on every DASS sub scale. 63% discontinues after reaching normal values at the total DASS scale. 97% discontinues after either reaching normal total DASS scores or making an important improvement since their start. This findings suggest that patients tend to continue this program until they feel they can continue successfully on their own.

Conclusions

The '15Minutes4Me.com(1.0)' program creates coherent results with a significant progress of participants with a strong correlation. This makes this Ericksonian & Solution Focused program a valid tool for further investigation with microarray DNA techniques on human blood leukocyte. One blood sample should be taken at the start of the '15Minutes4Me.com(1.0)' program, another when the patient reaches normal values on the DASS-21 scale and one several weeks later after maintaining those normal DASS Values for several consecutive weeks in a row.

The average participant normalizes his DASS scores (indicating cure) within 28 days, the mean within 21 days. This means that the second blood sample for microarray DNA technology analysis should be taken at that moment of normalizing scores, after on average 3 to 4 weeks. The third blood sample should follow several weeks later.

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PSYCHOSOCIAL GENOMICS: A NEW VISION OF THE PSYCHOTHERAPEUTIC ARTS

Richard Hill

ABSTRACT

The new field of Psychosocial Genomics was first proposed by Ernest Rossi in 2002 and has been addressed progressively in articles, papers, books and conference presentations. The conceptual principle of studying the nature of activity-dependent and experience-dependent gene expression and neurogenesis, in relation to therapeutic practice, health and well being, is now being addressed by a range of research in a variety of disciplines. A current definition of psychosocial genomics is presented. Descriptions of current knowledge and understanding of gene expression at the biological level provide a platform for understanding the relationship between fundamental research and translational research applicable to psychotherapeutic practice. Rossi encourages the formation of International Psychosocial Genomics Research Groups for exploring these possibilities in academic institutions, government laboratories and health services, and professional medical and psychological societies. This paper extends an open call for further organization and research in this new vision for the development and facilitation of Psychosocial Genomics in translational research from laboratory to bedside.

A DEFINITION

Every new approach needs a clear definition that acts as a sound foundation for the future. It needs to be simple, in order to both provide inspiration, and to open doorways for future research and investigation. Ernest Rossi composed this statement (personal communication):

"Psychosocial genomics is the study of how psychological and social experience modulates gene expression in health and illness."

We now know that being awake, asleep or dreaming correlates with the expression of different genes in the brain and body (Ridley, 2003; Riberio et al., 2008). Likewise emotional states such as being in love, excited, depressed, stressed, relaxed, or lonely are also related to different patterns of gene expression (Schulkin et al., 1998; Dusek et al, 2008). Gene expression is critical to the process of synthesizing the proteins required to sustain life and living. Single stranded copies of specific lengths of our DNA are made through biochemical processes to create messenger RNA. The RNA moves out of the nucleus into the body of the cell where another set of biochemical processes translate the information encoded in the RNA into chains of amino acids that become the proteins our bodies employ in biological activities. Some genes are expressed in the regulated process of maintaining living requirements, while other genes are expressed in response to some experiential stimulus. This is called activity-dependent gene expression.

It now is possible to study how therapeutic hypnosis, meditation and many other psychological and spiritual rituals can not only stimulate or repress gene expression, but also change the way in which genes are expressed. Many researchers believe this is the answer to the many questions related to the so-called "Mind-Body Problem": How can the mind influence body? How does the placebo response work? Why do happy people live longer and healthier lives? How and why does psychological depression actually reduce the volume of neural tissue in the hippocampus of the brain?

INTRODUCTION

Ernest Rossi (2002, p, 13) was the first to publish a proposal for the "… creation of a new discipline, to be called 'psychosocial genomics,' to explore how the psychological dramas and social encounters of everyday life can turn on activity-dependent gene expression and neurogenesis in ways that optimize performance health and well being." For the past 40 years Rossi has been publishing prescient ideas, such as the relationship of genes and proteins to the experience of dreams (Rossi, 1972/1986/2000; 1973) and the deeper biological nature of mind-body healing (Rossi, 1986). Rossi's suggestions that mental activities, including therapeutic hypnosis, are influencing the biology of the client have proved to be inspiringly accurate. Rossi's ideas have finally been demonstrated through a recent study, titled "Pilot Study and GSEA analysis of gene expression following a session of therapeutic hypnosis" (Rossi et al., 2008; Atkinson et al., 2010). The pilot study was restricted to three participants and sought to establish in-principle protocols for larger and more rigorous experiments in the future. Whole blood samples were taken before, one hour after and 24 hours after the administration of a positive experience of therapeutic hypnosis known as the Creative Psychosocial Genomic Healing experience. Leucocytes (white blood cells) were analyzed for upregulated and downregulated gene expression. In his analysis of Rossi's subjects, Atkinson (2010) found that 15 immediate early

genes were expressed in the 1 hour samples and a further cascade of 77 genes was expressed in the 24 hour samples. Gene set enrichment analysis (GSEA) indicated an upregulation of genes characteristic of stem cell growth, a reduction in cellular oxidative stress, and a reduction in chronic inflammatory processes. The study is described in more detail below. Further studies are planned, and preliminary work, which has yet to be published, has been conducted with a larger group. These experiments have only become possible since the development of gene expression measuring techniques, such as the DNA Microarray (Shalon, et al., 1996) and others (Ronald et al., 2005; Yeung et al., 2003).

In an intellectual climate emphasizing evidence-based practice, we now have the methods and the tools to provide evidence for the benefit and effectiveness of mind-body therapeutics. There is ample observational and phenomenological reporting of the effectiveness of therapeutic hypnosis (Gonsalkorale et al., 2003; Cyna et al., 2004), as well as growing literature on the neuroscience of hypnosis (Dienes et al., 2009; McGeown et al., 2009). The analysis of gene expression provides further empirical evidence to confirm and validate these observations, strengthen the confidence of practitioners, and enhance the security of clients in the biological efficacy of treatment. I believe that Psychosocial Genomic research has the potential to produce the most important advances in the understanding of non-invasive health and healing practices since the neuroscience breakthroughs made possible by brain scanning techniques such as MRI and fMRI. Like the process of gene expression itself, there is not just a single action and reaction, but a cascading cycle of activity that expands into multiple and unexpected areas. Rossi's (2011, In Press) current thinking takes us further by seeking to draw closer together our complex biological processes and their interplay with the experience of consciousness.

THE HARD PROBLEM OF CONSCIOUSNESS AND PSYCHOTHERAPEUTICS

David Chalmers presented the "hard problem of consciousness" (1996) which asks how it is possible that physical processes (like gene expression and neuronal firing) give rise to a subjective experience. Chalmers called for extra ingredients to close this "explanatory gap". Through Psychosocial Genomic research we are discovering more about the intricate web of activity that both responds to consciousness and also creates consciousness. It is a bi-directional, dynamically linked cycle. Rossi has long since described the dynamics of a 4-stage creative cycle reflected throughout biology and philosophy (Rossi 2002). Rossi now draws our attention to experience- and activity-dependent gene expression as one of the extra ingredients Chalmers calls for. We can contemplate a Darwinian co-evolution of the processes of life and the qualia of consciousness (2011, In Press).

This lends supports to the view that the qualia of consciousness (our subjective feeling of a mental experience), is a co-evolving development of mind and the brain. It is entirely natural that biological and psychological processes have evolved together. The research literature outlines how life emerged from the non-biological chemical soup some 4 billion years ago, into an RNA reproducible world. This RNA world eventually evolved into a DNA world. DNA meant that biological information remained stable during replication and enabled the long process of Darwinian evolution (Gilbert, 1986; Yarus, 2010). The suggestion, however, of a co-evolution of the intangible qualia of consciousness is more difficult to grasp. It requires mathematical complexity theory and quantum concepts for a broad vision of the evolution of life and consciousness. Most simply stated: changes in DNA and gene expression are the fundamental basis of change in our biology, behavior and consciousness over time. This is fundamental evolutionary theory. Most important for therapeutic practice, there now is research that shows how mental and emotional activity and life experiences have a direct effect on gene expression, brain plasticity and the functioning of our

biology. This growing evidence of a bi-directional relationship between mind and body invites the conceptual possibility that physiological functions and the qualia of consciousness are co-existent and relational aspects of evolutionary change (Reichardt et al., 2000; Rossi et al., 2008; Yehuda et al., 2009; Gatt et al., 2010; Mcgowan & Szyf, 2010; Atkinson et al, 2010; Dusek et al., 2010; Coplan et al., 2011; Rossi, 2012).

Although we will not investigate that further here, it is relevant to reflect on therapeutic hypnosis as one of a number of practices that utilizes implicit and explicit mental activity on a regular basis to facilitate change, healing and well being. It is now clear that the activity and experience that plays out in a therapeutic session is engaging the whole body, on both the macro and micro levels. The details and evidence of these principles is why ongoing research is so important in helping us understand more clearly what actually happens to clients and to the therapist in the processes of therapy, in the activity of gene expression, and in the development of consciousness.

In a positive and practical light, Rossi (2004) describes the possibilities of our new knowledge of genes and gene expression:

Humanity is now at another profound but little understood transition: we are making epochal discoveries about how everyone can learn to turn their genes on and off to create a better brain, health and well-being throughout their lifetime…. Genes are inner resources that we can all learn to use in a creative manner in daily life to construct and reconstruct our brain and mind for optimum health and well being. (p. 15)

FROM NEUROSCIENCE TO CREATIVE PSYCHOSOCIAL GENOMICS.

Psychosocial genomics emerged from Rossi's mind in an environment of extraordinary thinkers and practitioners. Rossi's mentor, Milton Erickson had a clear sense that hypnosis was not "… the miraculous power of effecting therapeutic changes in the patient … (but) … that therapy results from an inner re-synthesis of the patient's behavior achieved by the patient himself." (Erickson, 1948/80 p. 35)

It may not be necessary for a practitioner to have a detailed knowledge of neuroscience or genomics, but it is important to have some general understanding of the connections between psychological activity,

gene expression and brain plasticity. The ability of the brain to generate and regenerate new neurons and synaptic connections, generally known as brain plasticity, is not a new idea. That the morphology and chemistry of the brain could be altered in response to experience was first established in 1964 (Bennett, et al., 1964; Hubel & Wiesel, 1965). That these changes could occur at any age was determined and confirmed by the 'mother' of brain plasticity, Marian Diamond in research on rats (Diamond et al., 1964). Research into the brain flourished in the 1990's after president George Bush declared the Decade of the Brain (Bush, 1990) which facilitated much needed funds and resources. It is not the knowledge of neuroscience that has been recent, but the wider application of this knowledge to human emotional health and well being. Psychotherapy and psychology have developed largely in response to the observation of behavior. The inner workings of the brain were poorly understood and often quite erroneous in prior eras, as evidenced by the scholarly acceptance of phrenology, the interpretation of the meaning of the shape and size of the head (Combe, 1853). Following the explosion of knowledge about the brain over the past two decades it may seem strange to imagine that training in many therapies that deal directly with the mind and brain do not require any formal or detailed knowledge of the brain itself. This is expected to change over time.

Neuroscience research has given us insight into the mental states engendered by hypnosis. Appreciation of the changes in how the brain is working during absorption and focused attention (Dienes et al., 2009; McGeown et al., 2009) allows the therapist to be more conscious of what is happening to the client. The simplified message from neurological research is that there is different activity in various parts of the brain depending on the needs or conditions of the moment. Any given state of mind relates directly to which brain areas are active.

Interpersonal Neurobiology defines the mind as "... a process that regulates the flow of energy and information." (Siegel, 2006, p. 248) This definition can be applied to the wider processes of biology, which is also a flow of energy and information. Rapparini (2010) presents a complementary view of the mind from the qualia perspective, "… the mind is the qualia of the brain's neural mechanisms, this is how the perceiver perceives himself, from within." (p. 169) Together these definitions embrace the relationship of mind to both the perception of living and the processes of living. If we think of the brain as not just the 100 billion neurons and 1 quadrillion synaptic connections in the skull, but also the kilometers of neural threads throughout the body, we begin to see the extent of the energy and information that flows through the body (Gilbey, 2007; Siegel, 2012). In addition to this we have an enormous array of messenger chemicals that are synthesized by the neural net and endocrine system, and carry their packages of information through the transport system of arteries and veins (Pert, 1997; Hadley, 2000). It is now becoming clear that the deeper biochemical processes of genes and gene expression, which underlie the synthesis of these proteins, need to be taken into account in any credible theory of mind.
Discussions about the relationship between behavior, human experience and the modulation of gene expression began to gain vigor at the turn of the century (Moore, 2001; Ridley, 2001), but were preceded by prescient thinkers such as Nobel

Laureate Eric Kandel who proposed in 1989 that "… changes in neuronal architecture (are) changes that result from learned alterations in gene expression" (Kandel, 2003, p. 103). Kandel later (1998) made his position clear:

Insofar as Psychotherapy or Counseling is effective and produces long-term changes in behavior, it presumably does so through learning, by producing changes in Gene Expression that alter the strength of synaptic connections and structural changes … of the brain . . . Stated simply, the regulation of gene expression by social factors makes all bodily functions, including all functions of the brain, susceptible to social influences. (p. 460)

In this environment of fertile supposition, Rossi proposed his new field of investigation and described the following research foci for Psychosocial Genomics (2002, p.4):

• Behavioral state-related gene expression: How behavioral states such as sleeping, dreaming, consciousness, vigilance, stress, emotional arousal, and depression are associated with different patterns of gene expression.

• The novelty-numinosum-neurogenesis effect: Highly motivated states of consciousness that can turn on and focus gene expression, protein synthesis, neurotransmitters, and neurogenesis in our daily creative work of building a better brain.

• Experience or activity-dependent genes generate the synthesis of proteins and neurogenesis in the brain that encodes new memory, learning and behavior.

• A lack of optimal gene expression and neurogenesis is now believed to be associated with psychological depression.

• Immediate early genes, behavioral state related gene expression and activity dependent gene expression are implicated as the processes that can facilitate a deep psychobiological approach to therapeutic hypnosis and holistic healing.

These elements and their systems are contributors to a pattern of energy and information flow that enables our biology to function. Despite the extraordinary wonder and complexity of the brain and the wider neural system, the single vital source from which all of these biological are regulated is the DNA. The process that enables this storehouse of information to function is gene expression. The complexity of activity that enables the genes in DNA to be expressed, and for an organism to thrive, may seem enormous. Yet, in the light of this complexity, the structure and function of DNA is surprisingly simple and elegant.

INFORMATION, CONSCIOUSNESS AND DNA

DNA is constructed from only 4 base chemicals called nucleotides – adenine, guanine, cytosine and thymine - which reside on the inner surface of two parallel, twisting, sugar and phosphate rails. DNA holds all the information required to produce and maintain a living organism. It is a storehouse of

information, an archive, a script. DNA is able to be reproduced either in specific sections (gene expression) or in whole (re-production). A gene is a specific and limited sequence of base pairs that replicate through a series of processes that involves the single-stranded RNA molecule. The entire DNA structure is called the genome. (Watson & Crick, 1953).

The structure of DNA is under continuous review. Recent research is suggesting that there are more than 4 base chemicals. It is already known that RNA utilises a different base chemical called uracil instead of thymine. Thymine is the same as uracil, but with an additional methyl group. Cells divide thousands of times during the lifetime of an organism, and certain genes may express millions of times, which helps us to understand the persistent nature of mutation as a driver of evolutionary change. Changes that occur in the DNA that are not inherited are called epigenetic. Epigenetic changes occur during the life cycle of the organism. An epigenetic methylation of cytosine creates a new base called 5-methyl-cytosine (Lister & Ecker, 2009). This new base can go through changes that alter the DNA sequence and therefore can alter the way the gene is expressed. A sixth variation occurs when a hydroxyl group and a methyl group is added to cytosine creating 5-hydroxymethylcytosine (Munzel et al., 2011). This change affects the activity of a gene and may halt gene expression, a phenomenon which biologists refer to as "silencing" the gene. There are two more suspected base constructs – 5-formycytosine and 5-carboxylcytosine - (Ito et al., 2011). Very recent research shows that our DNA and the protein creating RNA are not rigidly stable or constrained to just four bases. The changeable nature of the bases may indicate that organisms are designed to respond to their experience with biological changes that can occur during a single lifetime, not the epochs required by random genetic mutation. Nurture can have very direct and far-reaching effects on the structures that nature originally made available.

The human genome has some 3.2 billion base pairs (nucleotides) and roughly 20-23,000 protein coding genes that occupy less than 2% of the 23 chromosomes that make up the human genome (IHGSC, 2004). Interestingly, complexity of biological form is not necessarily related to the number of base pairs or genes. The single celled Amoeba Dubia has some 620 billion base pairs (Parfrey et al, 2008), the Californian poplar has some 45,555 genes (Tuskan et al., 2006) and the much-studied roundworm used in research, the 5cm Caenorhabditis elegans, has a similar number of genes to a human being (Linden, 2007). The key is how these genes are expressed. The human genome uses the majority of its DNA, called non-coding DNA, to create the tools to turn our 20,000+ protein coding genes into a massive variety and complexity of biological compounds and physiological structures that is still far from being thoroughly understood.

Rather than an ordered, methodical process, gene expression and the daily and hourly creation of our biological needs is a frantic, complex, self-organising, adaptive system. Our inner world is a constantly shifting biochemical milieu that triggers and stimulates the myriad of inner functions in response to the nature of our experience every moment of the day and night. Not only is this in response to physical experiences with the outside world or general needs of cell metabolism; gene expression is also prompted by emotional and mental processes that occur because of those experiences

(Rossi, 2002; Ridley, 2003). Research has shown that genes that enable brain plasticity and learning are expressed after rats are given a spatial learning exercise (Guzowski et al, 2001). Particular genes are expressed during sleep after meaningful experiences during the day, activating an interaction between the hippocampus and the cortex, in order to store memories and create associations with past experiences (Riberio et al., 2008). This, and more, is what can be expected to occur in a client following a successful therapeutic session.

THE PSYCHOLOGY OF ACTIVITY- AND EXPERIENCE-DEPENDENT GENE EXPRESSION

It may not be necessary to understand the molecular-genomic dynamics of activity- and experience-dependent gene expression at great depth, but, in the same way we have been learning about the mind-brain relationships, it is important to grasp the basic principles and some fundamental examples of mind-gene relationships.

Gene expression is not a simple one-step process. Throughout the body, the Earth's biosphere, the solar system and beyond, all systems are conceptually and mathematically complex. To create clarity, I have divided the activity of experience and qualia-dependent gene expression into 4 main categories:

1. Experience-dependent gene expression that is triggered by general activity of transcription factors in normal cellular processes e.g. immune response, energy production (e.g. ATP) and general, regular metabolic processes. (Jacob & Monod, 1963; Nestler & Hyman, 2002)

2. Expression that is epigenetically turned on in response to specific activity e.g. memory, clock genes (circadian and ultradian rhythms). (Levenson & Sweatt, 2005; Miller & Sweatt, 2007; Kyriacou & Hastings, 2010)

3. Expression that has been epigenetically silenced in response to experience and requires new experience to reframe, e.g. experiences of trauma, insecure attachment and negative environment resolved by new experience of therapy or general life experience. (McGowan & Kato, 2008; Mathews & Janusek, 2008; Yehuda et al., 2009)

4. Pre-expression effects in relation to enhancement of expression, e.g. enhancer RNA and post-expression to mRNA by disruption of translation, e.g. microRNA, RNA induced silencing complex (RISC) and RNA interference (RNAi). Included in this category of alterations to the structure and activity of RNA is alternative gene splicing. The first RNA copy of a gene at the transcription stage is "edited" by removing non-coding introns and joining exons to produce mRNA. Alternative versions of the gene are produced by excluding or including various exons which may then go on to translate into different proteins (Black, 2003; Gough, 2010; Kim et al., 2010; Mattick, 2010; Muthusamy et al., 2010; Ren, 2010; Shamron, 2010)

I am not going to expand on each of these categories here, since each requires further investigation. I will, however, briefly discuss some of the implications and applications of these processes to the practice of therapy and the deeper understanding of human behavior.

EPIGENETICS, PSYCHOTHERAPEUTIC EXPERIENCE AND EVOLUTION

Epigenetics has been a big topic in biology for the past decade, and is now entering the realm of psychology and other disciplines (Allis et al., 2007). Epigenetic processes result in chemical additions to DNA strands that change the way in which genes can be expressed. Epigenetic processes occur during an organism's lifetime in response to the environment and the experience of the individual and may be passed to subsequent generations of cells through cell division and replication. This is important in understanding how experiences like trauma and insecure attachment are "remembered" in the biology of a human being. The field of medicine maintained for much of the twentieth century that emotional issues were "just in the head". We now know that if something is happening to a person in the realm of their feelings, perceptions and/or behaviors, there may be a correlating change in their neurobiology, physiology, cellular function and gene expression. Exactly what changes occur, how broadly they affect the individual, and how permanent they are, is the subject of much investigation and a core interest of Psychosocial Genomics.

The detail of epigenetics can seem confusing, but the principal process is that sections of the DNA are "silenced" from expression by molecules such as methyl groups which bond tightly to the bases, so that the factors that normally trigger gene expression (transcription factors) are unable to attach to the area on the DNA that normally initiates gene expression (promoter regions). It has been found in research that neglectful mothering by female rats creates an epigenetic change to the DNA in the hippocampal region of the brains of her offspring. This change to the glucocorticoid receptor gene in the hippocampus of neglected rat pups renders them less able to handle stress, and inclines them to be anxious and hyper-vigilant (Meaney & Szyf, 2005).

This epigenentic change is, however, an adaptive survival mechanism. It may not make for a happy rat pup, but the increased vigilance and low sense of being nurtured makes the pup work harder to find the mother and to secure a teat for feeding. Research has shown that similar epigenetic influences occur in human beings following childhood abuse (McGowan et al., 2009) and social adversity in early life (McGowan & Szyf, 2010). What is even more intriguing is that if that pup is moved to the litter of a nurturing mother rat, the epigenetic change is reversed and the pup returns to being almost as unstressed as if it had been well-nurtured from the beginning (Meaney & Szyf, 2005). This is, quite simply, the way in which a negative experience alters us at a genomic level and how a positive experience, perhaps something like therapy or a good holiday, can rewrite an epigenetic memory and enable a mental reframing that resolves the issue. The positive experience triggers activity in the hippocampus that promotes reflection and meaning-making, especially at night and in dreams (Riberio et al., 2008).

Factors that promote neurogenesis and synaptogenesis are triggered to facilitate brain plasticity. The recent developments in understanding how to intervene in memory reconsolidation, in order to modify fearful memories, is directly related to gene expression (Feinstein, 2010; Ecker et al., 2012). When a memory is recalled it returns, for a short period of time, to a labile state which is subject to protein synthesis in order to reconsolidate the memory. At the same time, there are body-wide effects, such as increases or decreases in cortisols and inflammatory interleukins that can stimulate changes to the way that different cells function in the visceral tissue (Field et al., 2005).

In the practical sense, when a therapist works with a client, there are myriad processes that are triggered by the nature, quality and relevance of the therapeutic experience. Precisely what happens in the body after a therapeutic process that allows the mind to induce a change in the energy and information flow? This is the question that Rossi asks some decades ago. The International Psychosocial Genomics Research Group has been able to conduct the first study of therapeutic hypnosis that tests the relationship between psychotherapy, and experience- and activity-dependent gene expression.

THE INTERNATIONAL PSYCHO-SOCIAL GENOMICS RESEARCH GROUP (IPGRG)

After many years of vision, Rossi was finally able to conduct a pilot study to investigate the gene expression that followed a therapeutic hypnosis process. Rossi has long since developed and worked with a non-directive, implicit form of therapeutic hypnosis which had been described as the "mirror hand" process. For the purposes of this research Rossi officially named the process the Creative Psychosocial Genomic Healing Experience (CPGHE). This title highlights the creative, interactive, non-directive nature of the process, and its potential to create activity in the subject on all levels from mind to gene.

In association with colleagues at the University of Salerno, Italy, Rossi arranged for three volunteers to be tested. Peripheral blood was taken immediately before, within one hour and 24 hours after the CPGHE was conducted. The blood samples were treated according to the protocol recommended by the DNA Microarray analysis service at the University of Padua and in line with previous protocol in the literature.

It was found that within one hour, 15 early response genes were up-regulated. At 24 hours there was further cascade of activity in 77 genes. The experiment successfully established a working protocol, and demonstrated changes in activity-dependent gene expression (Rossi et al, 2008).

The DNA microarray data was further analyzed by other members of the research group, led by David Atkinson. The software program and gene database, GSEA, which is freely available on the web, was utilized to make a more detailed analysis of the gene expression that occurred. Results showed that there was beneficial change in relation to genes that regulate reduction in inflammatory processes, an up-regulation in genes that are characteristic of stem cell growth and an up-regulation of genes that reduce oxidative stress (Atkinson et al., 2010).

AN OPEN CALL FOR TRANSLATIONAL RESEARCH IN CREATIVE PSYCHOSOCIAL GENOMICS HEALING EXPERIENCE (CPGHE)

Rossi's (2011, In Press) Creative Psychosocial Genomics Healing Experience (CPGHE) now requires replication and extension. Equally, similar experiments that cover a wider range of patients, a wider range of cellular expression and a wider range of mind-body practices are needed to push the door open wider. The implications for the practicing therapist are wide-reaching. I suggest that this may be as important to future health and well being as the breakthroughs in neurobiology with the development of scanning techniques such as MRI and fMRI.

The CPGHE calls for interdisciplinary cooperation among three groups:
(1) cognitive-behavioral researchers, (2) DNA microarray laboratory researchers and (3) bioinformatics research teams that performs the computer software analyses of the meaning of the DNA microarray results (Rossi, 2005/2006; Rossi & Rossi, 2006).

Those with biological and/or genetic research training will see a plethora of future research questions that spring from the Pilot Study. If you are a practitioner, then it is important to gather data on the observed (therapist) and experienced (client) effectiveness of the CPGHE. The CPGHE is a protocol, framework and recording system to assist in research that can be done in the therapy setting. Reference material is available for review, including the rationale, administration, and scoring of the CPGHE protocol, and may be found in Rossi (2011, In Press). The protocol is also freely available online (Rossi/Neuroscienceresearch group, n.d.).

POTENTIAL DISCOVERIES IN FUTURE PSYCHOSOCIAL GENOMICS RESEARCH

Here are just a few examples of what might be found in future psychosocial genomics research

• Rossi (2005/2006) wrote about the possibilities of exploring therapeutic hypnosis with DNA Microarrays and suggested a number of candidate genes that might be expressed. Even within the limitations of the pilot study, changes in the expression levels of a number of those genes were, indeed, noted. Rossi also noted a gene called CYP-17. This family of genes, the cytochrome P family, also includes CYP-24 and CYP-27. These genes are genes that facilitate processes that are vital for health. CYP-17 acts to enable the production of testosterone and estrogen. . The CYP-24 and CYP-27 genes act in the cells of the liver and the kidneys to catabolise vitamin D into its active form, 1-hydroxy D. This active form is a transcription factor for more than 200 known genes that are vital for the immune system (Norman, 1985; Hollick, NEED DATE). It is fundamental in keeping cancerous cells from getting out of control (Garland et al., 2006) and is an important factor in depression and mental disorders (Stewart & Harani,

2010; Ganji et al., 2010). Complete processing of Vitamin D is necessary for multiple areas of health and well being. Experimental evidence that a mind-based therapeutic process is stimulating gene expression of CYP-17 and/or CYP-24 and CYP-27 would not only strengthen the evidence for mind-based therapy, but also integrate our understanding of the interplay between therapy and dietary nutrients. Is the stimulation of cytochrome P family genes one of the ways in which therapeutic hypnosis relieves depression and improves healing?

• Meta-analyses and literature reviews could bring disparate research together, to encourage cross-referencing of ideas, and to facilitate combined research that maximizes available facilities and budgets. Just a few examples of disparate research that could be collectively reviewed or analyzed: Hayashi and his team found that laughter can positively regulate gene expression in patients with Type 2 diabetes (Hayashi et al., 2006); Campbell and his team investigated the gene expression activity in response to stress and the impact on eating disorders (Campbell et al., 2010); and the surprising discovery by Kaptchuk and his team who tested the placebo response (Kaptchuk et al., 2010). The result was that even when the subjects knew that they were taking a completely inert substance, they improved. They were simply told that placebos have been shown in clinical studies to have a beneficial response "… through mind-body self-healing processes." With only that thought as inspiration, there was broad improvement in symptoms and quality of life. This raises the research question: What is the gene expression difference between hidden placebo and open placebo experiments?

• Research that incorporates a wide variety of non-invasive therapies and practices will benefit from, firstly, meta-studies, but ideally, concurrent studies to examine whatever variations and similarities exist between therapeutic practices and gene expression. Conceptual reviews of the link between successful psychotherapy and gene expression, as well as hypotheses for future investigation, are already appearing in the literature (Rossi, 2002; Feinstein & Church, 2010). Research that examines several different techniques in relation to different conditions and controls will lead to a clearer understanding of the similarities and variations between non-invasive therapies. Talk therapies; Eye Movement Desensitizing and Reprocessing (EMDR); Emotional Freedom Techniques (EFT); meditation; mindfulness; sympathetic attention; and even positive intention will benefit from comparative studies of their effects on gene expression.

• As gene expression measurement tools become more sophisticated it may be possible to develop a neuro-bio-geno-feedback process in which researchers can train activity-dependent genes to turn on and turn off. It is only by learning more about how the mind-to-body relationship works that we will be able to harness the benefits of our knowledge for better health and well-being. Those practicing meditation or deep mental focus may be achieving positive genetic effects already. How are they doing it? Can we make the best tools more accessible to a wider population? Is science just around the corner from completely revolutionizing the way we manage our body-to-mind-to-body integration?

It is currently difficult to pursue genetic research due to high costs and technical difficulties, but those barriers are falling

at a rapid rate. Fast and inexpensive gene expression analysis will open the door to many opportunities. Non-invasive treatments may be quickly assessed for their efficacy for a particular individual, permitting treatments that are tailored to the patient's genetic susceptibilities. When we are able to monitor gene expression in neuronal tissue, it may be possible to intervene in traumatic memory issues, such as PTSD, with therapies customized for a particular patient. Similar benefits may be possible for disrupted systems in complex neural circuits, as has been shown in subjects with OCD. There are, potentially, many areas of mental health where benefit might be achieved without having to impose risky drug interventions or undertake extended periods of treatment while hoping for behavioral change.

These are some of the important implications for further research:

1. The existing research has achieved the difficult task of establishing protocols and achieving, in principle, results that are in line with expectations. This means that further research has a foundation for success.

2. The opportunity to have a deep and lasting effects on the health of future generations, especially in the area of preventative health, might not only improve the 'happiness quotient' of our populations, but also free up large amounts of money and resources that would otherwise be spent treating preventable disease.

3. The potential for the integration of diverse academic and clinical fields into a dynamic complex of knowledge can redirect the central focus of healthcare back to the whole person.

4. Individual testing for the epigenetic effects of particular therapies brings us closer to the ideal of each individual being able to find the best and most effective protocols to stimulate their individual healing.

Psychosocial Genomics does not seek to be another disparate field of study, but rather an umbrella that arches over a wide range of investigation using interdisciplinary research. The challenge now is to make the vision a reality. We must push beyond the edges of current knowledge and understanding. This new frontier requires patience, because we must tread wisely into new territory. Yet we can approach it with enthusiasm because this field holds the nascent promise of a world in which we have the knowledge necessary to cure many chronic mind-body illnesses rather than just managing them.

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GUIDELINES FOR AUTHORS

1. The International Journal of Psychosocial and Cultural Genomics, Health and Consciousness Research (hereinafter the Journal) accepts for publication the following article types:

    a. Empirical manuscripts: researches based on original data
    b. Theoretical manuscripts (literature reviews and/or studies that propose original conceptual models and interpretive hypothesis);
    c. Reports (description based on criteria of psychological intervention).

2. The editorial staff also edits the following additional types of contribution:

    a. The point of view: articles of or interviews with prominent authors and/or institutional representatives in the topic of the issue.
    b. Translation of manuscripts published by the international literature that have a considerable scientific and professional interest.
    c. Reviews of books, articles, conferences, workshops in the field.
    d. Works published within other disciplines that have consequences for or connections with our studies
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3. The editorial staff preliminarily verifies the relevance of the manuscripts with the field of interest and the scientific line of the Journal, according to the paragraph 2 of these guidelines. The contributions considered relevant are then submitted to the evaluation of at least two independent referees expert in the topic of the manuscript. The referees are identified by the editors.

4. In order to evaluate a manuscript, the selected referees use a dedicated analysis grid. In the event of requesting changes, the grid and the manuscript are sent to the authors so that they can modify the manuscript as requested.

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    a. Empirical manuscripts: Novelty of the contribution and/or of the research hypothesis; clear identification of the objectives; relevance of the contribution in relation to the literature; conceptual framework; updated references; description of methodology; operational definition of the variables; methods of data processing; level of significance of results; implication for the intervention; syntax; intelligibility.
    b. Theoretical manuscripts: Novelty of the contribution; relevance of the contribution in relation to the literature; conceptual framework; description of the analysis method used; reasoning effectiveness; consistency; updated references; implication for the intervention; syntax; intelligibility.
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