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A Pilot Study of Positive Expectations and Focused Attention via a New Protocol for Optimizing Therapeutic Hypnosis and Psychotherapy Assessed with DNA Microarrays: The Creative Psychosocial Genomic Healing Experience

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Abstract

We extend the use of DNA microarrays to explore a new psychotherapeutic therapeutic protocol, The Creative Psychosocial Genomic Healing Experience, an easy-to-learn approach to facilitating therapeutic hypnosis, psychotherapy, rehabilitation, meditation, and pastoral counseling. This pilot study assessed the hypothesis that a top-down creatively oriented positive human experience can modulate gene expression on the molecular level. A DNA microarray data analysis of the white blood cells of three human subjects was performed immediately before, one hour after, and 24 hours after The Creative Psychosocial Genomic Healing Experience. We documented changes in the expression of 15 early response genes within one hour that apparently initiated a further cascade of 77 genes 24 hours later. This could provide the mind/molecular genomic foundation of new therapeutic models for optimizing human consciousness, health, and well being via therapeutic hypnosis, psychotherapy, pastoral counseling, and psychiatry. This proof-of-principle pilot study now requires cross validation with more subjects with a variety of diagnostic classifications to document the validity and reliability of using DNA microarrays to assess our new creative psychosocial genomic therapeutic protocol in a variety of cultures.

Introduction

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 (Dusek et al., 2008; Rossi, 2002, 2004, 2007; Rossi, 2005/2006). This has led to calls for further research on the pathways of psychotherapeutic processes on all levels from mind to gene (Abbott, 2008; Nestler, 2008; Rossi et al., 2006). In this pilot study we use DNA microarrays to assess a new therapeutic protocol, The Creative Psychosocial Genomic Healing Experience, as a relatively brief, easy-to-learn process for facilitating a wide range of therapeutic approaches such as therapeutic hypnosis, psychotherapy, rehabilitation, meditation, and pastoral counseling (Rossi, 2005/2006; Rossi and Rossi, 2008a and 2008b).

Material and Methods

Three highly susceptible hypnotic subjects, one male and two females, experienced therapeutic hypnosis following the new protocol called, “The Creative Psychosocial Genomic Healing Experience” established by Rossi (2004).

Peripheral blood, about 10 ml, was collected immediately before, within one hour of therapeutic treatment (the length of each treatment depended by the subject, but never went more than one hour). A total of 6 blood samples were employed to purify total RNA from leukocytes (the nucleated part of peripheral white blood cells) using the kit LeukoLOCK(TM) Total RNA system following the instruction supplied by the manufacture (Ambion, USA).

The amount of total RNA extracted from each blood sample was quantified using the NanoDrop ND-1000 photometer (Wilmington, DE). The purity of RNA samples was determined based on the ratio of spectrophotometric absorbance of the sample at 260 nm to that of 280 nm (A₂₆₀/A₂₈₀). However not all the RNA samples resulted pure enough (A₂₆₀/A₂₈₀ ratio of 1.8) to go through the microarray procedure, in fact protein contamination was still present in some of the samples. A further purification step by means of one phenol chloroform and one chloroform treatment was necessary to eliminate the contaminants.

Total RNA of each purified sample was further quantify using the NanoDrop apparatus and its integrity was ascertained by means of electrophoresis in agarose gels followed by ethidium bromide staining on the ribonucleic acid. Around 2.5 µg of 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 labelled using Amino Allyl cDNA Labeling Kit (Ambion, USA) following the manufacture instruction. Cy3/Cy5 was from Amersham Biosciences (Amersham, United Kingdom). Cy3/Cy5 dye incorporation into aRNA yielded incorporation rates of 30 to 60 dye molecules per 1000 nucleotides by spectrophotometric analysis, as requested by the manufacturer.

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). The laser power and the photomultiplier tube (PMT) were set between 70% and 80% of maximum. The excitation/emission settings were 543/570 nm for Cy3 and 633/670 nm for Cy5. After laser focusing and balancing of the two channels, scans were conducted at a resolution of 5 µm. For any scan, two separate 16-bit TIFF images were produced. Data were normalized by ScanArray Express using the LOWESS (Locally Weighted Regression Scatter Plot Smoothing; Cleveland, 1979) algorithm

After normalization, data from each slide were split in two, by using Microsoft Excel, since each probe is spotted twice. Thereafter, each spot value was considered to be independent and subjected to SAM (Significance Analysis of Microarrays; Tusher et al., 2001) analyses.

Since each comparison (S1/S1-1h, S2/S2-1h, S3/S3-1h,) was repeated at least twice, there were at least four values for each gene to be used in the SAM analyses.

Lists of genes with significant changes in expression among at least two experimental samples were identified at delta values that gave a false discovery rate (FDR) of 0%.

The possible role played by therapeutic hypnosis via “The Creative Psychosocial Genomic Healing Experience” on three subjects in up-regulating gene expression in leukocytes in the peripheral blood was investigated. To accomplish this, transcriptome changes were first monitored in untreated

subject, just after the treatment. The transcriptome variations were analysed by means of the Operon Human Genome Oligo Set Version 2.0 platforms. The effect of positive and creatively oriented therapeutic hypnosis immediately after the session was investigated using a direct comparison experimental design, with four repetitions of which one was a dye swap. As each probe was spotted twice on human array, the following SAM (Significance Analysis of Microarrays, Tusher et al., 2001) analysis was performed on a dataset of eight values for each gene.

Experimental Design

The effect of positively oriented therapeutic hypnosis via the administration of our Creative Psychosocial Genomics Healing Experience on gene transcription was monitored and the subject's measurements before the treatment were the common references. Each comparison (S1/S1-1h, S2/S2-1h; S3/S3-1h) was repeated at least twice, so that at least four values for each gene were used in the subsequent SAM analyses. The dataset was tested with SAM using a 'one class' study design. This analysis was carried out on the following three subgroups of data: S1/S1-1h, S2/S2-1h; S3/S3-1h. Using a delta of 0.2 and a median false discovery rate (FDR) of 0.00%, the analysis yielded 3207 genes as differentially expressed, six groups of genes were selected (the up- and down-regulated for each of the three subgroups of data). By crossing these data, it was possible to show the different up- and down-regulating effects.

Results

DNA microarray results on the three subjects in response to the therapeutic protocol within one hour after the treatment indicated that expression of 15 early response genes were up-regulated between 1.2 and 1.8 folds and no single gene was down-regulated. The list of the up-regulated genes is presented in Table one.

Table One: The Modulation of Gene Expression in Human Leukocytes By a New Protocol for Optimizing Therapeutic Hypnosis and Psychotherapy, "The Creative Psychosocial Genomic Healing Experience." The Gene Bank Accession, Gene Symbol, Gene Description and results in fold changes in up-regulated gene expression within one hour in response to therapeutic hypnosis.			
GB_Accession	Gene_Symbol	Description	Fold Change
AK057104	RPE65	Homo sapiens cDNA FLJ32542 fis, clone SMINT2000537 Sodium-coupled neutral amino acid transporter 2	1.777714817
NM_000329		Retinal pigment epithelium-specific protein (65kD)	1.664647867
AK055997		Homo sapiens cDNA FLJ31435 fis, clone NT2NE2000612 Ring Finger protein 165	1.617968537
AK056729		Homo sapiens cDNA FLJ32167 fis, clone PLACE6000450 Serpin B Proteinase Inibitor	1.596523872

NM_001074	UGT2B7	UDP glycosyltransferase 2 family, polypeptide B7	1.578875081
BC018130	F2RL1	Coagulation factor II (thrombin) receptor-like 1	1.506199199
NM_030824	FLJ14356	Hypothetical protein FLJ14356 zinc finger protein 442	1.469687506
NM_021122	FACL2	Fatty-acid-Coenzyme A ligase, long-chain 2	1.380622376
NM_004126	GNG11	Guanine nucleotide binding protein 11	1.372082479
NM_020980	AQP9	Aquaporin 9	1.366899043
NM_001186	BACH1	BTB and CNC homology 1, basic leucine zipper transcription factor 1	1.330834867
NM_002921	RGR	Retinal G protein coupled receptor	1.312291611
NM_024911	FLJ23091	Hypothetical protein FLJ23091 G protein-coupled receptor 177 Isoform 1 and Isoform 2	1.274787709
NM_000860	HPGD	Hydroxyprostaglandin dehydrogenase 15-(NAD)	1.224585804
NM_002110	HCK	Hemopoietic cell kinase	1.190732546

Discussion

The major limitation of this pilot study was the small number of subjects and the lack of appropriate controls. The lack of statistical power in this pilot study, due to the limited number of treated subjects, for example, does not enable us to assess the degree to which our results coincides with previous research supporting the hypothesis that therapeutic hypnosis could modulate gene expression (Lichtenberg et al., 2000, 2004). Well funded major research studies in psychiatric genetics utilizing DNA microarrays typically include as many as 2,000 to 20,000 subjects (Abbott, 2008). Our results, however, suggest that this pilot study provides documentation consistent with the hypothesis that our new therapeutic protocol, The Creative Psychosocial Genomic Healing Experience, may modulate gene expression in human white blood cells.

These preliminary findings suggest that positive expectation via The Creative Psychosocial Genomic Healing Experience, a new protocol for facilitating therapeutic hypnosis, generated the up-regulation of 15 early response genes within one hour.

The expression of these early response genes apparently initiated a larger cascade of gene expression 24 hours later. This unexpected finding may have important implications for the role of time and post-hypnotic suggestion in therapeutic hypnosis and many other psychological experiences. We are currently investigating the unexpected finding that a gene associated with bipolar disorder may be over expressed in response to our new protocol of therapeutic hypnosis.

We propose that the genes expressed in response to this new protocol may be related to a variety of functions associated with stress (Dusek et al. 2008), cognition and dreaming (Riberio et al., 2007), and psychiatric conditions (Tsankova et al., 2007). Suggestions for further research in this area have been recommended previously (Kustra et al., 2006; Nestler, 2008; Nuzzo, 2008).

We introduced a new therapeutic protocol, The Creative Psychosocial Genomic Healing Experience, for assessing the contribution of positive expectation, focused attention, therapeutic hypnosis, and psychotherapy to stress reduction and mind-body healing (Rossi, 1986/1993, 2002, 2004, 2007; Rossi and Rossi, 2008b). A salient feature of this new protocol is that it was found to

be more acceptable, with high face validity for the subjects who experienced it and the psychiatrist who administered it, as a positive therapeutic process in contrast to the more research oriented classical scales of measuring hypnotic susceptibility, which have been questioned regarding their appropriateness for therapeutic applications (Fromm & Shor, 1972 Wester and Sugarman, 2007).

Our new protocol, The Creative Psychosocial Genomic Healing Experience, however, now requires standardization in relation to the classical assessments of therapeutic hypnosis such as the Stanford Hypnotic Susceptibility Scale (Hilgard, 1965) and the Harvard Group Scale of Hypnotic Susceptibility (Shor and Orne, 1978) as well as the more general evaluation of consciousness and focused attention with objective measures such as the Tellegen Absorption Scale (Tellegen, 1981, 1982, 1992; Tellegen & Atkinson, 1974).

The successful utilization of this new Creative Psychosocial Genomic Healing Experience protocol for assessing research on humans with DNA microarrays may foreshadow a new psychosocial genomic paradigm to facilitate a “top-down” therapeutic approaches from mind to gene (Rossi, 1986/1993, 2007; Rossi and Rossi, 2008a & b). This could provide the mind/molecular genomic foundation of new therapeutic models for optimizing human consciousness, health, and well being via therapeutic hypnosis, psychotherapy, pastoral counseling, and psychiatry.

Conclusions

This pilot study assessed the hypothesis that a creatively oriented positive human experience of therapeutic hypnosis could modulate gene expression on the molecular level. We documented changes in the expression of 15 early response genes within one hour that apparently initiated a further a further cascade of 77 genes 24 hours later. This proof-of-principle pilot study now requires cross validation with more subjects to document the validity and reliability of using DNA microarrays to assess our therapeutic protocol, The Creative Psychosocial Genomic Healing Experience, as a new approach for facilitating therapeutic hypnosis, psychotherapy, rehabilitation, meditation, and pastoral counselling.

Acknowledgments

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References

- Abbott A. The brains of the Family. *Nature* 2008; 454, 7201:154-157.
- Dusek J, Hasan H, Wohlhueter A, Bhasin M, Zerbini L, Joseph M, Benson H, Libermann T. Genomic Counter-Stress Changes Induced by the Relaxation Response. *PLoS ONE* 2008; 3(7): e2576. doi:10.1371/journal.pone.0002576.
- Eisen M, Spellman P, Brown P, Botstein D. Cluster analysis and display of genome-wide expression patterns. *Proceedings of the National Academy of Sciences*, 1998;95:14863-14868.
- Fromm E, Shor R. *Hypnosis: Research Developments and Perspectives*. Chicago:Aldine Publishing, 1974.
- Hilgard, ER. *Hypnotic Susceptibility*. NewYork: Harcourt, Brace & World, 1965.

- Kustra R, Shioda R, Zhu M. A factor analysis model for functional genomics. *BMC Bioinformatics*, 2006; 7:216doi:10.1186/1471-2105-7-216.
- Lichtenberg P, Bachner-Melman R, Gritsenko I, Ebstein R. Exploratory association study between catechol-O-methyltransferase (COMT) high/low enzyme activity polymorphism and hypnotizability. *American J. Medical Genetics* 2000; 96:771-774.
- Lichtenberg P, Bachner-Melman R, Ebstein R, Crawford H. Hypnotic susceptibility: multidimensional relationships with Cloninger's Tridimensional Personality Questionnaire, COMT polymorphisms, absorption, and attentional characteristics. *International Journal Clinical of Experimental Hypnosis* 2004;52:47-72.
- Nestler E. A psychiatrist talks about finding answers that add up across all levels. *Nature* 2008; 451:1033.
- Nuzzo R. Nabbing suspicious SNPs. Scientists search the whole genome for clues to common diseases. *Science News* 2008;173:20-24.
- Ribeiro S, Xinwu S, Engelhard M, Zhou Y, Zhang H, Gervasoni D, Lin S, Wada K, Lemos N, Nicolelis M. Novel Experience Induces Persistent Sleep-dependent Plasticity in the Cortex but not in the Hippocampus. *Frontiers in Neuroscience* 2007;1:43–55.
- Rossi ER. *The Psychobiology of Mind-Body Healing: New Concepts of Therapeutic Hypnosis*. 2nd ed. New York: W. W. Norton, 1986.
- Rossi ER. *The Psychobiology of Gene Expression: Neuroscience and Neurogenesis in Therapeutic Hypnosis and the Healing Arts*. New York: W. W. Norton Professional Books, 2002.
- Rossi ER. (Translator & Editor, Salvador Iannotti: Saiannot@tin.it) *Discorso Tra Geni [Italian]. A Discourse with Our Genes: The Psychosocial and Cultural Genomics of Therapeutic Hypnosis and Psychotherapy*. Benevento, Italy: Editris SAS Press. [Available in Italian and English editions] New York: Zeig, Tucker, Theisen, 2004.
- Rossi ER. Prospects for Exploring the Molecular-Genomic Foundations of Therapeutic Hypnosis with DNA Microarrays. *American Journal of Clinical Hypnosis* 2005/2006;48:165-182.
- Rossi ER *The Breakout Heuristic: The New Neuroscience of Mirror Neurons, Consciousness and Creativity in Human Relationships: Selected Papers of Ernest Lawrence Rossi*. Phoenix, Arizona: The Milton H. Erickson Foundation Press, 2007.
- Rossi ER, Rossi, KL, Yount G, Cozzolino M, Iannotti S. *The Bioinformatics of Integrative Medical Insights: Proposals for an International PsychoSocial and Cultural Bioinformatics Project*. *Integrative Medicine Insights* 2006. <http://www.la-press.com/integmed.htm>.
- Rossi ER, Rossi, KL. Open Questions on Mind, Genes, Consciousness, and Behavior: The Circadian and Ultradian Rhythms of Art, Beauty, and Truth in Creativity. In Lloyd & Rossi (eds.) *Ultradian rhythms from molecule to mind: A new vision of life*. New York: Springer, 2008; 391-412.
- Rossi ER, Rossi KL. *The New Neuroscience of Psychotherapy, Therapeutic Hypnosis & Rehabilitation: A Creative Dialogue with Our Genes*. A Free Book on Line at www.ErnestRossi.com, 2008b.

- Shor R, Orne, E. Harvard Group Scale of Hypnotic Susceptibility. Palo Alto, California: Consulting Psychologists Press, 1978.
- Tellegen A. Practicing the two disciplines for relaxation and enlightenment: Comment on Qualls & Sheehan. *Journal of Experimental Psychology: General*, 1981;110:217-226.
- Tellegen A. Content categories: Absorption Items (Revised). Unpublished Manuscript, University of Minnesota, 1982, October 10.
- Tellegen, A. Note on structure and meaning of the MPQ Absorption scale. Unpublished Manuscript, University of Minnesota, 1992, August.
- Tellegen A, Atkinson G. Openness to absorbing and self-altering experiences ("absorption"), a trait related to hypnotic susceptibility. *Journal of Abnormal Psychology* 1974;83:268-277.
- Tsankova N, Renthal W, Kumar A, Nestler E. Epigenetic regulation in psychiatric disorders. *Nature Reviews Neuroscience* 2007;8:355-367.
- Tusher V, Tibshirani R, Chu G. Significance analysis of microarrays applied to the ionizing radiation response. *Proceedings of the National Academy of Science, U S A*, 2001;98(9):5116-5121.
- Wester W, Sugarman L. *Therapeutic Hypnosis with Children and Adolescents*. Bethel, CT: Crown House Publishing, 2007.

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AK056729		Homo sapiens cDNA FLJ32167 fis, clone PLACE6000450 Serpine B Proteinase Inhibitor	1,596523872
NM_001074	UGT2B7	UDP glycosyltransferase 2 family, polypeptide B7	1,578875081
BC018130	F2RL1	Coagulation factor II (thrombin) receptor-like 1	1,506199199
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