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Effects of Flotation REST on the Immune Response: T-Cells, B-Cells, Helper and Suppressor Cells

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Psychoneuroimmunology researchers are attempting to clarify how stress influences the immunologic process. It appears that assisted relaxation may be one psychological approach which may alter immune system responses. Peavy (cited in Achterberg, 1985) demonstrated the effects of biofeedback-assisted relaxation on immune function. When individuals, naive to immune function and the purpose of the study, learned to relax there was an enhancement effect on immunity. Achterberg (1985) observed that:

> Through many examples and varied processes, then, the filters that prevent direct mental access to the physical body can be lifted. Most of the ways have in common a means of either removing, or significantly altering, or even competing with the demands the external environment makes on the brain (p. 137).

Restricted Environmental Stimulation Therapy (REST) exposes the participant to an environment of reduced external stimuli for specified periods of time. Both chamber and flotation REST methodologies have been shown to produce relaxation responses (A. Barabasz, M. Barabasz,

Dyer, & Rather, 1992), while reducing distractibility (Zubek, 1969b). Participants become more open to new information (Suedfeld, 1980), and may enter the hypnotic state (Barabasz, 1990c,e).

Turner and Fine (1985b) investigated the effects of flotation REST assisted relaxation on the plasma levels of several hormones including cortisol, ACTH, luteinizing hormone (LH), and urinary cortisol. Subjects (n=12) were randomly assigned to either REST assisted relaxation or a similar procedure without REST. The experiment consisted of three phases: 1) the baseline phase of two 35 minute sessions, each one day apart; 2) the REST and nonREST relaxation phase of a 35 minute session for four consecutive days; and 3) the follow-up phase, four or five days after treatment, identical to the two session baseline procedure. Blood samples were obtained before and after sessions 1, 2, 5, and 8. There were significant changes in plasma cortisol and ACTH but not LH. Decreases in both cortisol and ACTH were demonstrated only in the REST assisted relaxation group. Cortisol showed a significant, progressive decrease across sessions in the REST group only while ACTH showed trends of decrease across sessions in both groups. REST assisted relaxation was associated with a specific decrease in activity of the pituitary-adrenal axis.

Later, Fine and Turner (1987) used a cross-over control design in which subjects used REST assisted relaxation with EMG biofeedback during one condition and control relaxation with EMG biofeedback in the other condition. Plasma cortisol was measured to compare the effects of treatment. Cortisol levels during the REST condition and the control condition were found to be significantly lower than baseline levels. However, there was no difference between conditions. It was concluded that perhaps both control relaxation and REST conditions were equally effective in producing physiological relaxation.

Recent research on voluntary immunomodulation emphasizes strategies such as hypnosis, relaxation, imagery, and biofeedback (Hall, 1989, p. 85). Often, the suggestion to relax is combined with vivid imagery. Janoski and Kugler (1987) compared the effects of relaxation alone and relaxation with imagery on selective indices of the immune function. The study attempted to determine whether either or both major neuroendocrine (pituitary-adrenocorticoid and sympathetic

adrenomedullary) pathways were associated with immune function. Subjects (n=28) were prescreened and identified as high in the capacity for imaginative involvement which is related to hypnotic capacity (Barabasz, 1984). Subjects were randomly assigned to one of three conditions: 1) progressive muscle relaxation, 2) progressive muscle relaxation and imagery for enhanced immune function, and 3) a vigilance task control. Salivary immunoglobulin A (SIgA), an index of immune function, was significantly enhanced by relaxation alone.

The relationship between REST and hypnosis has recently become a focus of study (Barabasz, 1988, 1990c,e, 1992). Hypnotizability, typically a very stable trait (Barabasz, 1982) provides a new way of explaining subjects' responses to REST. Hypnotizability can be enhanced by chamber REST (A. Barabasz, 1982; A. Barabasz & M. Barabasz, 1989) but not flotation REST (Barabasz & Kaplan, 1989). However, flotation REST elicits spontaneous hypnosis in subjects who already possess hypnotic talent (Barabasz, 1990c,e).

Experimentally-induced cold pressor pain has been used to test for the possible occurrence of spontaneous hypnosis in flotation REST (Barabasz, 1990c,e). High hypnotizables (n=10) and low hypnotizables (n=10) were exposed to three conditions in random order. Condition A consisted of a flotation REST session where subjects remained in the tank without a hypnotic induction. After 45 minutes of REST, cold pressor pain data were obtained. In Condition B, subjects were seated comfortably and exposed to a hypnotic induction during administration of cold pressor pain. In Condition C (controls), subjects were treated as in Condition B but no hypnotic induction was used. Results showed significant pain reduction effects for both REST (A) and hypnosis (B) conditions compared with the control condition for both high and low hypnotizables. However, pain reduction was significantly greater for subjects capable of high levels of hypnosis compared to the lows. Consistent with A. Barabasz, M. Barabasz, Dyer, and Rather (1990), all subjects reported the flotation REST sessions to be relaxing. However, only the high hypnotizables added hypnosis-like descriptions of the experience. It was hypothesized that while pain reduction for lows may be due to relaxation, the greatly enhanced pain reduction for highs may be due to both relaxation and hypnosis. Consistent with Hilgard's

(1977) neodissociation conceptualization of hypnosis and J. R. Hilgard's (1974) imaginative involvement findings, Barabasz (1990c) hypothesized that flotation REST produces spontaneous hypnosis in subjects known to have hypnotic talent.

The purpose of this investigation was to determine the effects of flotation REST on the immune response. It was considered important to control for subjects' hypnotizability level.

Method

Subjects

Ss (n=44) consisted of student volunteers (18-56 years of age) enrolled at large rural university located in the Pacific Northwest United States. Subsequent to a prescreening medical questionnaire, subjects were oriented to hypnosis, maximized in hypnotizability, and divided into high or low hypnotizability groups on the basis of Stanford Hypnotic Susceptibility Scale: Form C (Weitzenhoffer & Hilgard, 1962) scores (highs >7, lows <5). No subjects reported blood disorders, malignancy, auto immune disorder, active virus, allergy, immuno-suppressant medication, or life styles that would prohibit blood donation.

Instrumentation

A self-administered computerized version of the Symptom Checklist 90 R (SCL-90-R) (Derogatis, 1977) was employed. Ninety items are rated on a 5-point scale of distress from "not at all" to "extremely." Scores are obtained for 10 primary symptom dimensions (somatization, obsessive compulsive, interpersonal sensitivity, depression, anxiety, hostility, phobic anxiety, paranoid ideation, and psychoticism). Three global indices of distress are also calculated: Global Severity Index (GSI), Positive Symptom Distress Index (PSI); and Positive Symptom Total (PST). Raw scores were transformed into T-scores using non psychiatric

norms and a "cut off" GSI score of 70 was established in order to exclude subjects who reported life stresses which might be likely to confound immune function data.

Assay Process

Peripheral blood was obtained by venipuncture. Each of three samples per subject consisted of 16cc of blood. Assays were performed at the Microbiology Laboratory at Washington State University. The Ficoll-Paque method for in-vitro isolation of lymphocytes was used on fresh blood samples. White blood cell counts were performed by standard hematologic techniques (Jackson & Warner, 1986). Due to the complex nature of T- and B-cell function, a pilot study was conducted to determine which subsets of lymphocytes were likely to manifest proliferation. The use of monoclonal antibodies (Becton-Dickinson, 1986) permitted targeting and marking of T-, B-, and T-cell subsets of helper and suppressor cells. The cell staining procedure followed the protocol for Simultaneous Two Color Enumeration of T-Helper/Inducer and T-Cytotoxic/Suppressor cells, and Human T- and B-cells in peripheral blood (Becton-Dickinson, 1986). The standardized modification technique for multiple plate samples was used. Flow cytometry was employed to facilitate analysis.

Apparatus

A Becton-Dickinson FACS 440 flow cytometry system was used to detect electronic and optical signals from cells which were illuminated by laser light. Simultaneous multiparameter analyses of surface characteristics, cell volume, and cell size were obtained (Jackson & Warner, 1986). Surface antigens present on retrieved peripheral blood lymphocytes were bound and labelled by specific mouse monoclonal antibodies, developed to detect T-cells, B-cells, helper T-cells, and suppressor T-cells. Fluorescent dyes, conjugated to the antibodies, were distinguished by flow cytometry to facilitate identification and

enumeration of specified cells. Color dot plot graphs were generated which yielded percentages of T-, B-, and T-cell subsets of helper and suppressor cells.

Skin conductance response (SCR) was determined for each subject immediately prior to each venipuncture blood sampling using standardized procedure (Barabasz, 1977, p. 131-132). SCR was monitored by using a Lafayette Instruments 76100-30 Barabasz Desensitization Quantifier. A "cutoff" SCR was established during the pilot study to determine the arousal SCR stress level associated with venipuncture which would likely confound immune function. Two subjects with measurements beyond cutoff were excluded from the study.

The REST tank, Floatarium Brand SWS, is an enclosed, sound-insulated, fiberglass tub containing a 24 cm. deep solution of tap water and epsom salts ($MgSO_4$, 20% solution). The solution was maintained at 34.5° ± .2° C. Ventilation was provided by a silent positive pressure system. Subjects wore 28 decibel attenuating wax earplugs and floated supine with ears submerged. Subjects were monitored throughout the session via a built-in microphone. Subjects showered immediately before and after sessions.

Procedure

Subjects were randomly assigned, within high and low hypnotizability groups, to either flotation REST or control conditions. All subjects received written pre-treatment instructions which included refraining from excessive exercise, caffeine, dieting, and being abstinent from alcohol and nicotine. Subjects were asked to get adequate sleep within 24 hours prior to scheduled sessions. A pre-treatment blood sample was obtained from all subjects immediately prior to the first session or at the first scheduled meeting with control subjects. Each 16 cc. blood sample included two heparinized tubes, one EDTA tube, and one serum tube. Each subject was given a code number identifying their blood sampling series so blood analyses could be conducted blind with respect to treatment group. SCR was measured during each blood

drawing to account for the impact of venipuncture-related anxiety (Olness, 1988). All subjects were first given an orientation to the immune response via a short videotaped presentation which gave an overview of the major components and functions of the human immune system.

Subjects in the REST group received an orientation to the flotation tank (Suedfeld, 1980) and were told that they would be monitored throughout each session. Prior to preparation for the one-hour flotation tank experience, subjects took the SCL-90-R, self-administered computerized version. The one-hour flotation REST experience was uninterrupted with the exception that subject inquiries were responded to by the experimenter at the start of each session. Blood samples were taken at the end of the session. The second session followed in one week. Prior to REST Treatment 2 the SCL-90-R was again administered. A third blood sampling and SCR measure followed the REST session.

Control subjects were scheduled and run at the same times used for REST treatment subjects. At the first session, control subjects took the SCL-90-R and pretreatment blood samples were obtained. Blood samples were again obtained one hour later. These subjects were then scheduled for a second session one week later, during which the SCL-90-R was readministered and third blood sample taken.

After the second REST treatment and second control session, an independent post-experimental inquiry was conducted. This semi-structured interview was intended to evaluate subjects' experiences and identify extraneous factors that may have impacted immune function (i.e., development of life stressors or infectious conditions during the course of the study). In addition, comments from subjects in the REST group were evaluated to provide data on the possible occurrence of spontaneous hypnosis (Barabasz, 1990c,e).

A two-week post-experimental follow-up telephone contact also was made with all subjects to determine whether an active infection was developing during participation in the study. This procedure was intended to eliminate data from subjects whose immune systems may have been impacted by responses to infections.

Results

The data were analyzed by a 2 (Treatment = REST, Control) X 2 (Hypnotizability = Hi, Lo) X 3 (Time = Pre-treatment, Post-treatment 1, Post-treatment 2) ANOVA with repeated measures. Due to wide variations in pre-treatment measures of immune response ANCOVAs were also used to analyze between subjects data. An alpha level of .10 was selected as suggested by Orne and Scheibe (1964) for data of the kind generated in this study. Significant between subjects results emerged from this analysis for the following: 1) T-cells: Treatment X Hypnotizability $F(1,30) = 4.39$, $p = 0.45$; and 2) Absolute T-4 cells X Hypnotizability $F(1,27) = 3.45$, $p = .074$. Post hoc follow-up revealed a significantly lower percentage of T-cells for REST subjects high in hypnotizability compared to REST subjects low in hypnotizability and Control subjects low in hypnotizability. Post hoc tests for Absolute T-4 cells revealed that, regardless of treatment, subjects low in hypnotizability demonstrated significantly greater T-4 cell counts than subjects high in hypnotizability. Significant within subjects results were found for the following indicants: 1) B-cells: Hypnotizability X Time $F(2,62) = 2.96$, $p = .059$; 2) H-cells: Treatment X time $F(2,62) = 2.82$, $p = 0.67$; 3) Absolute T-4 cells: Time $F(2,56) = 2.61$, $p = .082$, Treatment X time $F(2,56) = 3.54$, $p = .036$; Treatment X Hypnotizability X Time $F(2,56 = 2.50$, $p = 0.91$; 4) H/S ratio Treatment X hypnotizability X Time $F(2,62) = 3.17$, $p = .049$. Post hoc comparisons showed that, regardless of treatment, there was a significantly greater percentage of B-cells in subjects high in hypnotizability from Pre-treatment to Post-treatment 2. Subjects low in hypnotizability demonstrated a significant decrease in percentage of B-cells from Pre-treatment to Post-treatment 2. Follow-up analysis for the two-way interaction of Treatment and Time for percentage of H-cells showed a significant increase for REST subjects from Pre-treatment to Post-treatment 2. Control subjects decreased in percentage of H-cells from Pre-treatment to Post-treatment, although not significantly. Overall, subjects showed a decrease in Absolute T-4 cells from Pre-treatment to Post-treatment 2. Follow-up analyses revealed that REST subjects decreased in Absolute T-4 cells from Pre-treatment to Post-treatment 1 and Post-treatment 2. REST subjects high in hypnotizability had the greatest decrease from Pre-treatment to Post-treatment 2. The three way interaction follow-up analysis for H/S ratio revealed that REST subjects high in hypnotizability showed a significant increase from Post-treatment 1 to Post-treatment 2. See Tables 23-1, 23-2, and 23-3.

Discussion

This was the first study to use monoclonal antibodies to mark and enumerate lymphocytes as a measure of immune function in Ss exposed to REST. Our findings suggest that flotation REST may enhance immune function. However, the data must be regarded as very preliminary. There was considerable variability in immune function data, despite prescreening of subjects to meet the criteria of a healthy, normal, non-stressed population. Apparent changes in a direction of enhancement or suppression must be interpreted with caution. This reflects both the homeostatic nature of the human body and the more subtle effects of flotation REST in altering the physiology of the immune response. It is noteworthy that in Peavy's (cit. Archterberg, 1985) study of biofeedback assisted relaxation and immune function, subjects selected were those under high levels of stress manifesting low levels of immunity as measured by white blood cell counts and neutrophil function. When these highly stressed subjects learned to relax, there was a direct but selective effect on immunity. Neutrophil function increased following treatment, suggesting relaxation may enhance immune function toward homeostasis in an immune suppressed sample. Turner and Fine (1987) suggested that since REST produced a decrease in pituitary-adrenal axis activity its effects seem to be more than a simple response to relaxation. They also observed that REST is associated with the release of endogenous opioids. These findings are consistent with the current psychoneuroimmunology research which implicates endorphins, interferon, interlukins, and

Table 23-1

B-Cell and T-Cell Results for High and Low Hypnotizables

Indicancy	Tx/Hyn	+/-	N	Mean Rank	Z	P (2 tailed)
B-Cell	REST HI	INC	8	(6.4)	-1.60	0.10
		DEC	3	(5.0)		
			11			
	REST LO	INC	3	(5.0)	-0.88	0.37
		DEC	6	(5.0)		
			9			
	Control HI	INC	4	(5.5)	-0.05	0.95
		DEC	5	(4.6)		
			9			
	Control LO	INC	5	(3.6)	-0.00	1.00
		DEC	3	(6.0)		
			8			
T-Cell	REST HI	INC	7	(6.1)	-0.88	0.37
		DEC	4	(5.7)		
			11			
	REST LO	INC	5	(4.8)	-0.17	0.85
		DEC	5	(5.2)		
			10			
	Control HI	INC	7	(5.0)	-1.48	0.13
		DEC	2	(5.0)		
			9			
	Control LO	INC	2	(3.0)	-1.68	0.09
		DEC	6	(5.0)		
			8			

Table 23-2

H-Cell and S-Cell Results for High and Low Hypnotizables

Indicants	Tx/Hyn	+/-	N	Mean Rank	Z	P (2 tailed)
H-Cell	REST HI	INC	7	(5.4)	-0.44	0.65
		DEC	4	(7.0)		
			11			
	REST LO	INC	5	(5.0)	-0.29	0.76
		DEC	4	(5.0)		
			9			
	Control HI	INC	5	(4.8)	-0.17	0.85
		DEC	4	(5.2)		
			9			
	Control LO	INC	1	(1.0)	-2.38	0.02
		DEC	7	(5.0)		
			8			
S-Cell	REST HI	INC	3	(6.6)	-1.15	0.24
		DEC	8	(5.7)		
			11			
	REST LO	INC	5	(4.3)	-1.12	0.26
		DEC	6	(5.3)		
			11			
	Control HI	INC	2	(6.5)	-1.12	0.26
		DEC	7	(4.5)		
			9			
	Control LO	INC	6	(5.5)	-2.10	0.03
		DEC	2	(1.5)		
			8			

Table 23-3

Absolute T-4 Cells and H/S Ratio Results for High and Low Hypnotizables

Indicates	Tx/Hyn	+/-	N	Mean Rank	Z	P (2 tailed)
Absolute T4	REST HI	INC	0	(0.0)	-2.52	0.01
		DEC	8	(4.5)		
			8			
	REST LO	INC	2	(6.5)	-1.12	0.26
		DEC	7	(4.5)		
			9			
	Control HI	INC	5	(5.6)	-0.65	0.51
		DEC	4	(4.2)		
			9			
	Control LO	INC	2	(5.5)	-0.98	0.32
		DEC	6	(4.1)		
			8			
H/S Ratio	REST HI	INC	7	(5.5)	-0.53	0.59
		DEC	4	(6.7)		
			11			
	REST LO	INC	6	(5.1)	-1.00	0.31
		DEC	3	(4.6)		
			9			
	Control HI	INC	6	(4.3)	-1.12	0.26
		DEC	2	(5.0)		
			8			
	Control LO	INC	0	(0.0)	-2.20	0.02
		DEC	6	(3.5)		
			6			

peptides as chemical messengers in the two-way communication feedback loop of the body-mind.

The findings reported here used hypnotizability as a blocking variable in an attempt to better understand REST effects. Another aspect of the research, which exposed additional subjects to hypnotic inductions and self-hypnosis, demonstrated that hypnosis enhanced immune function results well beyond those produced by REST. This data (Ruzyla-Smith, A. Barabasz, M. Barabasz, & Warner, in preparation) will be reported elsewhere.

The production of a significantly higher percentage of T-cells by the low hypnotizability REST subjects compared to high hypnotizability REST subjects is difficult to explain. In an attempt to explain the findings of significantly enhanced hypnotizability following six hours of chamber REST, it was hypothesized that "REST forces the organism to focus, perhaps as seldom before, on internally generated imaginal activity" (Barabasz, 1982; p. 162). Barabasz (1982) conceptualized this response as a dissociative reaction which serves to maintain neuronal integration in the organization of brain functions. Sean Mee (see Chapter 2) elaborated on Barabasz's conceptualization of the phenomena within Hilgard's (1977a,b) neo-dissociation theory. During the postexperimental inquiry for the present study, all flotation REST subjects reported subjective feelings of deep relaxation. However, only the high hypnotizables described enhanced perceptual experiences. The highs noted, "I was more aware of what was going on in my body", "Images were coming, going, and changing a lot", "Pictures came to me in vivid colors", "I went on an exciting (imaginary) adventure... the time in the tank seemed like only a few minutes".

The second author has been attempting to determine whether the lack of specific suggestions in flotation REST with high hypnotizables may cause paradoxical hyper arousal as suggested by the arousing perceptual experiences described above, the occurrence of reduced EEG alpha, and increased electrodermal activity during mid-REST (A. Barabasz & M. Barabasz, 1985). Perhaps the absence of a positive descriptive suggestion to enhance the immune response, produced a hyperarousal state in the high hypnotizables thus mitigating REST relaxation effects and, thereby, precluding immune response

enhancement. As recently demonstrated (Barabasz, 1990e) (also see Chapter 5), flotation REST elicits spontaneous hypnosis in high hypnotizables. The highs in the present study may not have shown immunoenhancement because the relaxation effects of flotation REST, experienced by the normal population, were not manifested by the highs because of their active engagement in satisfying and stimulating imaginative involvements (Barabasz, 1984). The findings of a significantly lower percentage of T-cells in the high hypnotizable REST subjects seems to support this speculation and suggests further investigation. Assigning subjects high in hypnotic susceptibility to a REST group with a positive suggestion to enhance the immune response and then comparing results to a REST only group would be an initial step towards understanding the interactive effect of REST and hypnotizability. Perhaps the low hypnotizables, who were incapable of entering hypnosis, responded to the relaxation effects of the tank showing cell counts consistent with immunoenhancement.

It would seem that researchers of voluntary immunomodulation should be sure to consider hypnotizability and study the effects of relaxation combined with imagery on immune function enhancement. This research should employ imagery that "is consistent, at least metaphorically, with actual immunological functioning" (Hall, 1989, p. 87). The selection of immune function indices should be related to the accuracy of imagery used as well as the choice of a component that shows reactivity to psychological interventions. Several questions regarding immunological measures remain including: 1) When should post-treatment samples be taken? 2) What individual differences may be linked to enhancement or suppression of the immune response? and 3) What effect does invasiveness of obtaining samples have on the results?

Explorations of mind/body interactions are multifaceted and often complex. In seeking the truth, nonsignificant findings also add to this growing field of knowledge. The significant findings, observed trends, and nonsignificant findings suggest the following areas for future research.

1. Comparing Ss, grouped by hypnotizability, in flotation REST with hypnosis to enhance immunological function with other flotation REST Ss without hypnosis.

2. Comparing stressed and/or immune suppressed Ss, with Ss meeting criteria for nonstressed and/or immune suppressed using the above treatments.

Undertaking this type of study may have far reaching multidisciplinary implications in light of advances being made in the fields of health psychology, psychoneurology, neuroendocrinology, immunology, and REST.

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