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EFFECTS OF IMAGERY VERSUS RELAXATION
ON WHITE BLOOD CELL ACTIVITY

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EFFECTS OF IMAGERY VERSUS RELAXATION ON WHITE BLOOD CELL ACTIVITY

By

Sarah B. Whitcher

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ABSTRACT

EFFECTS OF IMAGERY VERSUS RELAXATION
ON WHITE BLOOD CELL ACTIVITY

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An A-B-A experimental design with replications was used to test the hypothesis that imagery of neutrophil motility is associated with increased activity of the cells *in vitro* whereas relaxation is not related to increased activity. Three groups of four subjects participated in the experiment. One group was trained in progressive relaxation and imagery of neutrophils. A second group was trained in progressive relaxation alone. Training took place after a baseline measure. A third group constituted an untreated control. Subjects participated in the experimental conditions (25 minutes of imagery/relaxation (imagery group) or 25 minutes of progressive relaxation (relaxation group)) three weeks after a baseline condition. Control subjects participated in two baseline conditions.

Blood samples were obtained by venepuncture before and after the two conditions and analyzed for adherence (a variable reflecting activity of cells *in vivo*). Imagery and relaxation groups were run before the control

group. During this phase of the experiment, experimenters remained blind to ownership of blood samples. A follow-up measure (one blood sample) was taken one week after the experimental condition for imagery and relaxation groups.

Results indicated that while adherence increased significantly during the experiment for all subjects, no differences were found among conditions, suggesting that the treatments had no effect on neutrophil function. The most reliable finding was the presence of marked individual differences in adherence levels during baseline which decreased over time, presumably due to an habituation effect. In addition, high adherence levels were associated with small changes in adherence, suggesting a ceiling effect, and making results more difficult to interpret. Results are discussed in terms of studies of the physiological effects of cognitive and physical stressors.

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CHAPTER I

INTRODUCTION

The emergence of the field of "psychoneuroimmunology" (Ader, 1981) reflects growing evidence of central nervous system mediation in immune system processes. Stress has been shown to have immunosuppressive effects in both animals (Monjan, 1981) and humans (Jemmot & Locke, 1984). Other psychological factors such as learning (Ader, 1981), hypnosis (Bowers & Kelly, 1979), imagery (Achterberg & Lawlis, 1978), meditation, and relaxation (Wallace & Benson, 1972), may also be associated with alterations in immune system processes. The purpose of this experiment was to study changes in one aspect of host resistance, neutrophil adherence, as a function of relaxation and imagery techniques in healthy subjects.

About neutrophils

Neutrophils are a type of white blood cell important in host resistance. Their suitability for psychoneuroimmunological research rests upon several factors. First, the effects of psychological factors can

be readily observed due to their rapid response to stimuli (they have an 8 hour lifespan, and are programmed to act within this period, as opposed to monocytes and lymphocytes which live for months to years and respond slowly) (William, Beutle, Erslec, & Wayne, 1977). Secondly, they are functionally homogeneous and are the most common type of white blood cell (in contrast to lymphocytes which are relatively rare and have many different functions), and thus isolating procedures are straight-forward. Third, highly accurate and reliable procedures for measuring neutrophil function are available.

Neutrophils, like other white blood cells, are produced in the bone marrow, and are found in the circulatory system when in their resting state. Neutrophils go through a metamorphosis when stimulated by a substance called a "chemotactic factor" (CF) (There are several naturally occuring chemotactic factors, some produced by bacteria as a by-product of metabolism, some produced by the immune system).

When stimulated by CF, neutrophils change from a spherical shape to a bipolar configuration (See Figure 1). In addition the adhesiveness of the cellular membrane increases for margination (adherence to the blood vessel wall), and migration toward the site of infection or injury. This migration occurs along a CF

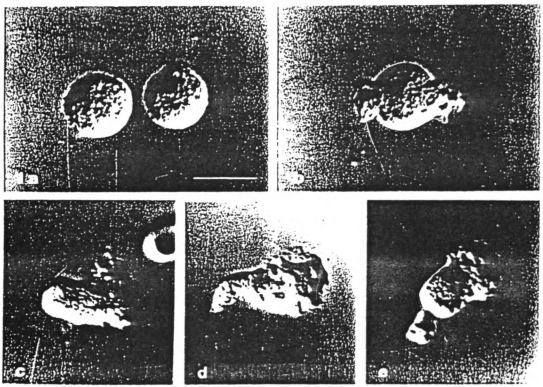


FIGURE 1

Electron micrograph of activation states in human neutrophils

1a: Cells in resting state

b: Initial shape change

c: Development of bi-polar configuration

d: Definition of head (left) and tail (right) regions

e: Cell migrating

gradient of increasing concentration (Smith, Hollers, Patrick, & Hassett, 1979). Once neutrophils reach the site of infection, they pass between the cells of the vascular endothelium into the surrounding tissue (emigrate), where they engulf bacteria by phagocytosis (Quie & Cates, 1979).

The adherent properties of the neutrophil membrane are critical to its ability to marginate, migrate, and emigrate; individuals with a rare disease in which their neutrophils lack adherence are at risk of serious and often fatal infectious disease (Smith, Hollers, Dupre, Goldman, & Lord, 1972).

Stress and resistance to infectious disease

In a recent review, Jemmot & Locke (1984) concluded that psychosocial variables may play a role in modulating the human immune response to infectious disease. Stress is the most commonly investigated psychosocial variable in these studies, and there is considerable agreement that stress affects immunocompetence in animals (Monjan, 1981). However, the results of animal studies are not conclusive in developing models for humans (Jemmot & Locke, 1984) particularly stress models, due to individual differences in responses to stress (Lazarus, 1966). As Jemmot & Locke (1984) point out, most people exposed to infectious disease do not become ill.

Early research on the relationship between stress and susceptibility to infectious disease in humans focused on studying the effects of stress hypothesized to be a result of major life changes. In a study of susceptibility to tuberculosis, Holmes, Hawkins, Bowermen, Clarke, & Joffe (1957) found an increase in life changes 1 to 2 years prior to hospitalization.

Later studies confirmed a weak but consistent relationship between life changes and susceptibility to respiratory illness in employees (Hinkle, 1974), children (Meyer & Haggerty, 1962), and college students (Jacobs, Spilkin, & Norman, 1969).

Bartrop and his associates (Bartrop, Luckhurst, Lazarus, Kiloh, & Penny, 1977), found that bereavement is associated with reduced T-lymphocyte response to mitogenic substances (substances that cause cell division, and thus proliferation). Natural killer cell activity also appears to be suppressed in subjects who are undergoing significant life stress and who are poorly defended psychologically (Locke, Kraus, Leserman, Hurst, Heisel, & Williams, in press).

Neutrophil phagocytosis is depressed in stressed infants (Wright, Ank, Herbert, & Stiehm, 1975), and in sleep deprived individuals (Palmbad, Bjorn, Wasserman, & Akerstedt, 1981). Studies from the manned Apollo

spaceflights (Fisher, Daniels, Levin, Kinzey, Cobb, & Ritzman, 1972) found alterations in lymphocyte counts and responses to mitogenic stimulation after splashdown.

A recent study by Dorian, Garfinkel, Brown, Stone, Gladman, & Keystone (1982) demonstrated the immunosuppressive effects of stress in a population of students. Elevated numbers of T and B lymphocytes and impaired mitogen responsiveness were found in a high stress group as compared to controls. These effects normalized after the stress was removed.

Research indicates that the immunosuppressive effect of stress may be mediated through central nervous system substances. According to Monjan (1981), animal studies indicate that "there are areas of the CNS where catecholamine activity is altered in response to stress." (Monjan, 1981, p.205) Stein, Schiavi, and Camerino (1976) found that hypothalamic lesions alter cell-mediated immune responses. Elevations in glucocorticoids and epinephrine (a catecholamine) impair neutrophil adherence and migration (MacGregor, 1977).

Effects of other psychosocial factors on immune processes

Controlled studies of psychosocial factors other than stress in relation to infectious disease are few. Conditioning, hypnosis, relaxation and imagery have received some attention as factors affecting immune

responses in general.

Ader & Cohen (1975) utilized a taste aversion learning paradigm to behaviorally condition immunosuppression, and later applied this principle to delay the development of murine systemic lupus erythematosis in mice (Ader & Cohen, 1982). In addition, the paradigm has been used to behaviorally condition suppression of a graft-versus-host response in rats (Bovbjerg, Ader, & Cohen, 1982). A related study by Smith and McDaniel (1983) demonstrated that the tuberculin skin reaction (Mantoux reaction) could be suppressed in tuberculin positive subjects who expected their reactions to be negative.

Hypnosis has been used to treat immune-related disorders, such as warts (Sinclair Gieben & Chalmers, 1959), and allergic dermatitis (Ikemi & Nakagawa, 1962). Relaxation training is an effective adjunctive treatment in asthma (Creer, 1982) and arthritis (Achterberg-Lawlis, 1982).

Imagery and relaxation are important components of a treatment program for advanced cancer patients developed by Simonton (Simonton, Simonton, & Creighton, 1978). In this treatment program patients receive conventional medical treatment in addition to group and individual psychotherapy, and training in progressive relaxation and imagery. Patients image their white blood cells as

potent and aggressive, and their cancer cells as weak and ineffectual. The imagery, called "specific imagery" or "disease imagery", is used to give the therapist information about their patients's will to live, and is thought to facilitate development of hope and a sense of control over the disease (Holden, 1978). Some proponents of the "Simonton Technique" believe the imagery has a direct effect on the disease process.

Achterberg & Lawlis (1978) utilized self-reports of patients' disease imagery to predict the course of cancer in advanced cancer patients. In a follow-up study of 126 patients who had elected to participate in the Simonton treatment program, imagery (as quantified through an objective rating system based on imagery content) was a better predictor of survival rate than the blood chemistry techniques conventionally used for this purpose (Achterberg, Achterberg, Simonton & Simonton, 1977).

Imagery and neutrophil function studies

A series of experiments were conducted through the Psychiatry and Anatomy Departments at Michigan State Univerisity to study the correspondence between content of imagery and physiological changes in healthy subjects under controlled conditions (Schneider, Smith, & Whitcher, Note 1). Three experiments were conducted in which the content of imagery instructions (e.g., imaging

white blood cells as active or inactive) was the independent variable. Neutrophil adherence and total white blood cell count were the dependent variables. Adherence tends to correlate with lowered white cell counts, as the circulating pool is reduced with margination. Thus it was hypothesized that different patterns of neutrophil adherence and total white blood count would be found with different imagery instructions, and that changes would occur during imagery and not during a baseline condition.

Results suggested that there was a correspondence between the content of imagery instructions and activity of the cells. In Experiment 1, subjects were instructed to image their neutrophils becoming more active, marginating and emigrating. In these subjects, significant reductions in both count and adherence were found. The reduced adherence was hypothesized post hoc to be a consequence of the reduction in count; the less adherent cells were available to venepuncture, while the more adherent cells had marginated.

In Experiment 2, designed to test the above post hoc hypothesis, the instructions were to image the cell activating but not marginating. It was hypothesized that the imagery would reduce margination. Increased adherence was found in most of the 25 subjects participating in this experiment. There were no changes

in count.

In Experiment 3, de-activation and non-margination were imaged. Results indicated that adherence had decreased in 12 out of 14 subjects, while in two subjects marked increases were found. There were no significant changes in count.

According to Schneider et. al., the results suggest that since both increases and decreases in adherence of neutrophils were found under conditions of relaxation and imagery, and because the content of imagery was the only variable manipulated, it is the nature of the imagery rather than relaxation that affects neutrophil function.

Other reported findings in this research are first, an association between subjects' scores on an imagery rating instrument similar to the instrument designed by Achterberg & Lawlis, (1978), and second, what appears to be a training effect. Some subjects' neutrophils showed changes in adherence before the experimental session in which the imagery was used. These changes apparently occurred as a function of the training procedure.

Schneider et. al. (Note 1) concludes that although further research is necessary, the results suggest that 1) imagery/relaxation is related to changes in neutrophil function, 2) that minor alterations in imagery correspond to significant shifts in neutrophil function, and 3) that self-reported imagery is significantly associated with

these changes.

Statement of the problem

The purpose of the current experiment was first, to replicate the Schneider et. al. (Note 1) findings of alterations in neutrophil function in subjects trained in imagery/relaxation.

A second objective was to test the hypothesis that imagery is directly associated with changes in neutrophil function. If the content of imagery instructions is a critical factor in the changes observed on a cellular level, relaxation without imagery should be associated with no change in neutrophil function, or with a different pattern of change.

In addition, a follow-up condition was added to study the effects of time on changes in neutrophil adherence. In the imagery and neutrophil function studies (Schneider, Note 1), alterations in adherence were observed prior to the experimental session. These changes may have taken place during the training and may have lasted from two to three weeks. The follow-up measure was included to assess the duration of any changes. No specific hypothesis was made regarding the outcome of this measure.

Research has shown that individuals who are hypnotizable are more likely to respond to treatments of physical disorders using suggestion (Bowers & Kelly, 1979). Bowers & Kelly suggest that the "healing impact of communications" (p. 495) (e.g., hypnotic suggestions) holds primarily for high hypnotizable subjects whose "receiver characteristics" allow a "transduction" of information from the semantics of suggestion to physiological outcome.

If specific content of imagery is associated with neutrophil function, high-hypnotizable subjects should show a greater magnitude of change than low-hypnotizables. An instrument to assess hypnotizability was included in the present study. However, since only eight subjects were used, no specific hypotheses were made reagrding the relationship of this measure to outcome.

Hypotheses

Hypotheses tested in the current experiment were as follows:

[1] In subjects trained in imagery/relaxation, changes in neutrophil function will be found during an imagery session.

[2] Patterns of changes in neutrophil function for

subjects trained in imagery/relaxation will differ from patterns found in subjects trained in relaxation alone.

CHAPTER II

METHOD

Design

An A-B-A single case study design was used in this experiment. Experimental subjects completed baseline, experimental, and follow-up conditions. Control subjects completed two baseline conditions and one follow-up condition. Thus, for experimental subjects, there were five measures of neutrophil function in the experiment; pre and post baseline, a 25 minute neutral activity, before and after a 25 minute experimental session, and the follow-up measure. The two treatment conditions were progressive relaxation (Relaxation Group) and guided imagery of neutrophils (Imagery Group). The imagery condition was based on the imagery training procedure used by Schneider (Schneider, Smith, & Whitcher, Note 1).

Experimental subjects were matched on sex and hypnotizability (as assessed by the Harvard Group Scale of Hypnotic Susceptibility Shor & Orne, 1962) before intensive training in the experimental treatment techniques, and following the baseline condition. They were not matched on baseline level of neutrophil function

as change in neutrophil function with the experimental treatments was of primary interest. For control subjects, there were five measures of neutrophil function; before and after two baseline conditions, and one follow-up measure.

Control subjects had five measures of neutrophil function; before and after two baseline conditions, and one follow-up measure. Control subjects were not matched, as the control condition was run subsequently to the experiment in which the effects of imagery versus relaxation was assessesd.

Blindness to results was maintained during the experimental condition through the help of a laboratory technician who took blood samples of subjects and maintained a code regarding their identities unknown to the experimenters. Two subjects participated in the Experimental condition simultaneously, and the blood samples were identified only by the code. A co-experimenter, JN, a student at the College of Osteopathic Medicine, participated in training the subjects and in analysis of blood samples. These procedures were not used with control subjects.

Subjects

Subjects for the experimental condition (imagery versus relaxation), were volunteers and were recruited through the College of Human Medicine (CHM) and the College of Osteopathic Medicine (COM). Recruitment information was distributed to all students in the first and second year classes of COM and CHM in the form of a brochure. The brochure stated that the experiment was an investigation of psychological influences on white blood cell function. Potential subjects were asked to contact one of the experimenters by phone for more information. During the phone conversation with an experimenter, the time commitment was discussed, subjects were screened for major health problems, and scheduled to come to the laboratory for the Baseline measure. No mention was made of the content of the imagery that would be used. Subjects were advised that they would not have a choice in assignment to the two treatment conditions, but that they could be trained in and tested in the other treatment after the conclusion of the experiment.

All experimental subjects were friends or aquaintances of JN, the co-experimenter. Of the 14 subjects expressing interest, 10 participated in the baseline condition. Eight of these 10 completed the experiment. One subject dropped out due to circumstances unrelated to the experiment, and one subject contracted

an infection resulting in abnormal neutrophil function. Subjects who dropped out were not replaced, leaving two males and two females in each treatment condition. Subjects received no monetary compensation for their participation.

Control subjects were recruited through a leaflet posted on bulletin boards in class areas of COM and CHM students. Control subjects were paid for their participation.

Procedure: Imagery and Relaxation groups

Baseline

Subjects were scheduled to come to the laboratory for the baseline measure in the morning, to control for the different levels of stress subjects might encounter during the day. Subjects were introduced to the experimenters and lab technician, shown around the lab, and questions were answered. Subjects were asked to fill out a questionnaire regarding health behaviors as a neutral task to equate subjects' activities during the baseline condition.

To control for the effects that rushing to the appointment might have on blood function, a blood sample was taken no sooner than 10 minutes after arrival. A second blood sample was taken between 20 and 25 minutes

after the first blood sample. This second sample served to control for possible psychological (psychosocial stress) and physiological (immune reaction due to introduction of skin cells into the blood via the needle) responses to venepuncture.

Hypnotizability assessment

The outcome of psychophysiological experiments may be significantly dependent on the hypnotizability of subjects (Bowers & Kelly, 1979). To control for this source of variability, subjects in the two groups were matched on the basis of their scores on the Harvard Group Scale of Hypnotic Susceptibility (HGSHS), Form A (Shor & Orne, 1962). The Harvard Group Scale is an adaptation of the individually administered Stanford Hypnotic Susceptibility Scale (SHSS) (Bentler & Hilgard, 1963), which can be group administered with self-report scoring. Research findings indicate that the norms derived from the adapted scale are congruent with norms derived from the SHSS (Bentler & Hilgard, 1963; Shor & Orne, 1963).

While the instrument is designed for group administration, it was individually administered, as it was considered advantageous to utilize uniform instructions delivered by an experienced hypnotist. To accomplish this, subjects individually listened to tape-recorded instructions through headphones in a quiet

room, and filled out the questionnaire. The recording had previously been made by an experienced hypnotist.

Subjects obtained scores ranging from 5 to 10, the mean score was 6.6, comparable to the reported mean of 7 in college aged populations. Two matched groups of four subjects each were formed for the experimental conditions (relaxation and imagery); two subject with scores of 10 were yoked, along with two sets of 2 subjects with scores of 6, and one set with a score of 4 and 5. Control subjects were not assessed for hypnotizability.

Training Procedure

Imagery Group

Training of the imagery group closely paralleled training used in the experiments conducted by Schneider et. al. (Note 1). Subjects in the imagery group participated in three group training sessions (education, relaxation and general imagery, specific imagery). Sessions were scheduled one week apart. In addition subjects practiced individually at home, and were seen for an individual training session in the research setting before the experimental session.

Education session. General information about neutrophil function was presented and slides of neutrophils were shown. An electron microscope was used

to prepare these slides which provided three-dimensional pictures of neutrophils in various stages of activation (inactive, migration, emigration, phagocytosis), to facilitate development of subjects' visual imagery of the cells.

Meichenbaum & Turk (1976) found in research on the use of imagery in control of chronic pain that subjects were able to use imagery to control pain more effectively when they were given didactic material on pain mechanisms. Therefore, education about cell function was thought to be important in the imagery training procedure used by Schneider (Note 1) and in the current experiment.

Guided imagery and relaxation session. Subjects were introduced to principles of relaxation and imagery. Uses of these techniques in pain control and systematic desensitization were presented along with theories regarding reasons for their effectiveness. Subjects practiced progressive relaxation according to the procedure outlined by Luthe (1969). A guided imagery experience was included in the instructions for relaxation. The sound of ocean waves was played on a tape recorder to facilitate relaxation and screen out extraneous noise.

Specific imagery. Subjects were trained in imagery of neutrophils based on the work of Simonton et. al. (1978) and Achterberg & Lawlis (1978) who maintain that

emotional and symbolic imagery is more "effective" in creating physiological changes than concrete imagery. Results of previous experiments (Schneider et. al, Note 1), indicating that subjects are able to control the activity of their neutrophils, was presented. Following the didactic material, subjects practiced specific imagery of neutrophils after progressive relaxation. They were given instructions during the guided imagery to imagine their cells activating, marginating and emigrating (See Appendix A for verbatim instructions). The sound of ocean waves was played as before. After the imagery, subjects drew pictures of their images to facilitate group discussion and sharing of imagery techniques.

Relaxation group

This training procedure was designed to control for the effects of visual imagery on white blood cell behavior. Subjects participated in three group training sessions in which there was no practice in visual imagery or imagery of neutrophils. Instead, the sessions focused on progressive relaxation. Subjects practiced relaxation at home, and met with the experimenter for a practice session before the experiment, as in the imagery training. Again, training sessions were scheduled one week apart.

Education. Information about relaxation and trancendental meditation (a technique similar to relaxation according to Benson (1962)) was presented, along with evidence that these techniques have beneficial effects on physiological functioning. Benson's (1962) notion of the relaxation response formed a theoretical background for integrating research findings. The immune system and neutrophil function was not discussed.

Relaxation training I. Didactic material presented the previous week was reviewed and subjects practiced progressive relaxation according to the Luthe (1969) procedure. The sound of ocean waves was played as in the imagery training program.

Relaxation training II. Subjects practiced progressive relaxation to the sound of ocean waves (See Appendix B for verbatim instructions). After the relaxation, subjects discussed their techniques for relaxing during the session, and prior experiences in relaxation and/or meditation.

Individual home practice

While uniformity of treatment was desired, Meichenbaum and Turk (1976) suggest that imagery techniques are more effective when subjects have a choice of imagery used in techniques. Several subjects had extensive experience in relaxation and found the

instructions distracting. Therefore, subjects were given a choice of tapes for home practice of the experimental technique with and without instructions. Tapes without instuctions had the sound of ocean waves in the background. Tapes with instructions had the sound of ocean waves and the instructions to be used in the experimental condition. Subjects were asked to practice relaxing at home for at least three twenty-minute sessions over three days using these tapes.

Laboratory practice sessions

Subjects practiced their techniques in the research setting individually before participating in the experimental session. All components of the practice session were the same as the experimental session except no blood was taken. Subjects imaged or relaxed using the tapes they had used to practice at home. After the session, they discussed their experience with the experimenter who made suggestions for modifications, to increase the effectiveness of their imagery or relaxation. Subjects who had difficulty were encouraged to practice further before the experimental session.

Experimental sessions

Two subjects participated in the experiment at the same time to prevent experimenter bias. The two blood samples were identified by codes known to the laboratory

technician alone, the experimenters remaining blind to the treatment condition associated with each sample. Subjects were scheduled for early morning hours, to control for different levels of stress during daily activities.

Subjects were greeted by the technician and escorted to a private room. They were given a tape recorder and headphone for listening to the tape. The technician obtained a blood sample 10 minutes after the subjects' arrival. The second blood sample was obtained 25 minutes later, after the termination of subjects' imagery or relaxation.

Follow-up session

Subjects returned one week following the experimental session for a single blood sample to assess possible long term changes in neutrophil function concomitant with training in imagery and relaxation. Such changes have been observed in previous research. In addition, this measure was designed to assess the reversibility of the treatment conditions.

Procedure: Control group

The baseline condition for imagery and relaxation groups was repeated on two consecutive weeks for control subjects. Control subjects were given minimal

information about the research. They were told that the goal of the project was to collect baseline data on neutrophil function in medical students. Subjects arrived at the laboratory between 7 and 7:30 am and had one sample taken by a medical technologist no less than 10 minutes after arrival. Subjects were asked to bring recreational reading material for reading during the 25 minute interval between samples. A second sample was taken after this interval. A final follow-up sample was obtained three weeks after the first baseline session.

Neutrophil function measures

Blood samples were obtained by venepuncture. Vacutainer collection tubes were utilized. 5 ml. of blood was obtained in each venepuncture. A white count and differential were performed according to standard procedure as outlined below. A measure of neutrophil adherence with whole blood was performed according to the procedure outlined by McGregor (1977).

Count

White cell count and differential were performed by first counting the total number of white cells in a solution of 20 microliters of whole blood with 10ml of buffered saline solution with a Coulter counter. Two counts were made of each sample to ensure reliability.

Differentials for the control groups were determined by making a blood smear on a slide and counting the number of neutrophils versus other types of white blood cells through a microscope. These differentials were performed by a medical technologist. Differentials for the relaxation and imagery groups were determined using the procedure below.

Two slides were made of each sample on a Larc spinner. These slides were stained and counted with a Honeywell automated cell scanner to determine the differential (% neutrophils vs. % other types of white blood cells). 100 white cells per slide were counted. Because of the expected reliability of the automated cell scanner counting procedure, and because of economic considerations, only one of the two slides made was counted. Differentials for the control group were performed by a medical technologist using standard microscope procedure.

Reliabilities were calculated by generating correlations among the cell counts for each of three slides collapsing across subjects. Reliabilities were lower than expected for imagery and relaxation groups; .69 for baseline, .82 for the experimental session, and .63 for follow-up phases of the experimental conditions. For the control condition, reliabilities were .48, .70,

and .37 for the two baseline measures and follow-up.

Nylon wool adherence

This adherence assay is an adaptation of the procedure outlined by McGregor (1977) and has been found to have excellent reliability. As previously stated, the membrane of a neutrophil increases in adhesiveness with increasing activation. Neutrophils have an affinity for nylon wool and will adhere to nylon wool fibers in proportions depending on their level of activation. Thus the differential count of whole blood before being passed through nylon wool can be compared to the differential count after, and a value for adherence can be obtained.

In the procedure used in this experiment, eighty mg. of nylon wool was packed into a .25 ml space in the tip of a tuberculin syringe with an 18 G needle (referred to here as a column). For each sample, 3 columns were placed in test tubes and 1.5 ml. of blood was transfered from the vacutainer to the column with a Pasteur pipette. The blood was allowed to flow through the nylon wool for 5 minutes. Differential count was performed before and after the blood passed through the nylon wool columns, and the following formula was used to calculate neutrophil adherence:

$$100\% - \left[\frac{\text{PMN/ml in effluent sample}}{\text{PMN/ml in original sample}} \times 100\right] = \%\ \text{PMN ADHERING}$$

Three columns were used for the differential. A count

was made of the effluent sample from each column with a coulter counter, and two slides were prepared with a Larc spinner. One of the two slides was counted with a Honeywell automated slide scanner. 50 cells were counted, as most of the neutrophils adhered to the nylon wool (mean adherence was 70%). The adherence for a given sample was defined as the average value for the three columns.

CHAPTER III

RESULTS

Baseline

Descriptive statistics for imagery, relaxation, and control groups during baseline are shown in Table 1. Adherence values were higher than those found in the Schneider et. al. experiments. A different measure of adherence was used in these experiments (Note 2), thus the results of the current experiment are not directly comparable.

A group by trial repeated measures analysis of variance showed no group by trial interaction effects, and no main effects (See Appendix C for Analysis of Variance summary tables.) However, as shown in Figure 2, mean adherence for imagery cases decreased during baseline while an increase was found in relaxation cases. Control cases showed little change. A repeated measures analysis of variance comparing the results for the imagery and relaxation groups was significant ($F(1,1)=7.96$, $p<.05$) (See Appendix C). These results are important as they suggest that neutrophil adherence may change in response to unknown factors in a way that looks like a treatment effect when no treatment has been administered.

TABLE 1

Descriptive Statistics

Adherence (in percent neutrophils adhering) for relaxation, imagery, and control groups.

		SESSION 1				SESSION 2			
		Pre	Post	M		Pre	Post	M	
IMAG-ERY (N=4)	M	75.69	62.05	68.87	13.64	74.52	87.31	80.91	-12.79
	(SD)	(10.03)	(11.39)			(14.46)	(3.93)		
RELAX-ATION (N=4)	M	66.20	71.62	68.91	-5.42	79.94	84.16	82.05	-4.22
	(SD)	(12.05)	(8.74)			(6.50)	(5.15)		
CON-TROL (N=4)	M	65.93	60.02	62.97	5.91	64.51	71.24	67.87	-6.73
	(SD)	(13.77)	(20.50)			(17.58)	(13.86)		

		FOLLOW-UP
IMAGERY (N=4)	M	78.40
	(SD)	(6.59)
RELAX-ATION (N=4)	M	83.11
	(SD)	(4.94)
CON-TROL (N=4)	M	76.9
	(SD)	(6.5)

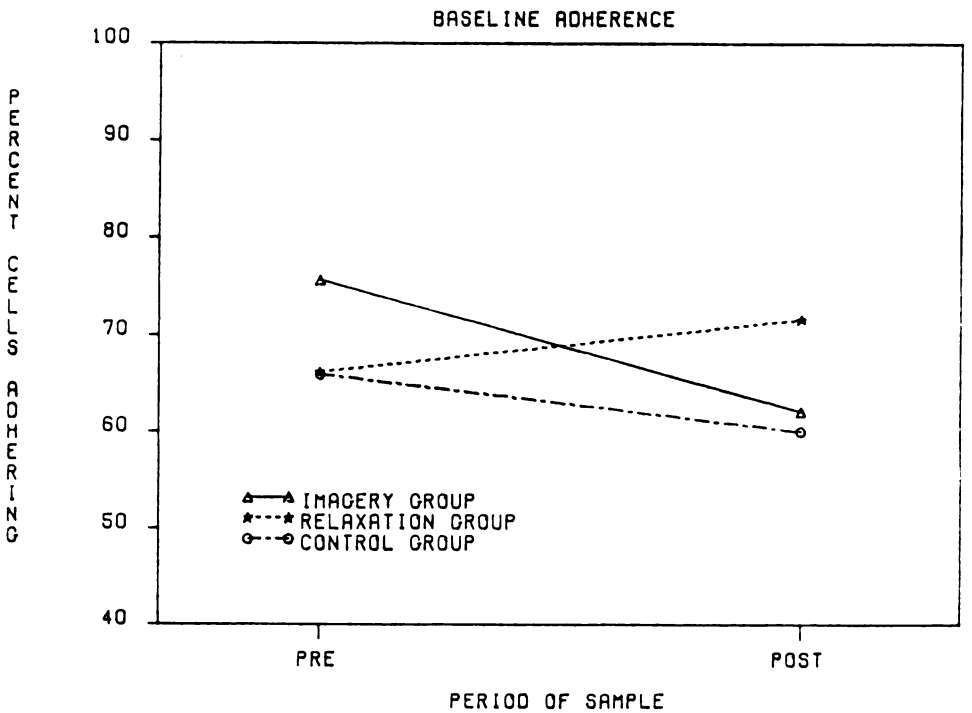


FIGURE 2

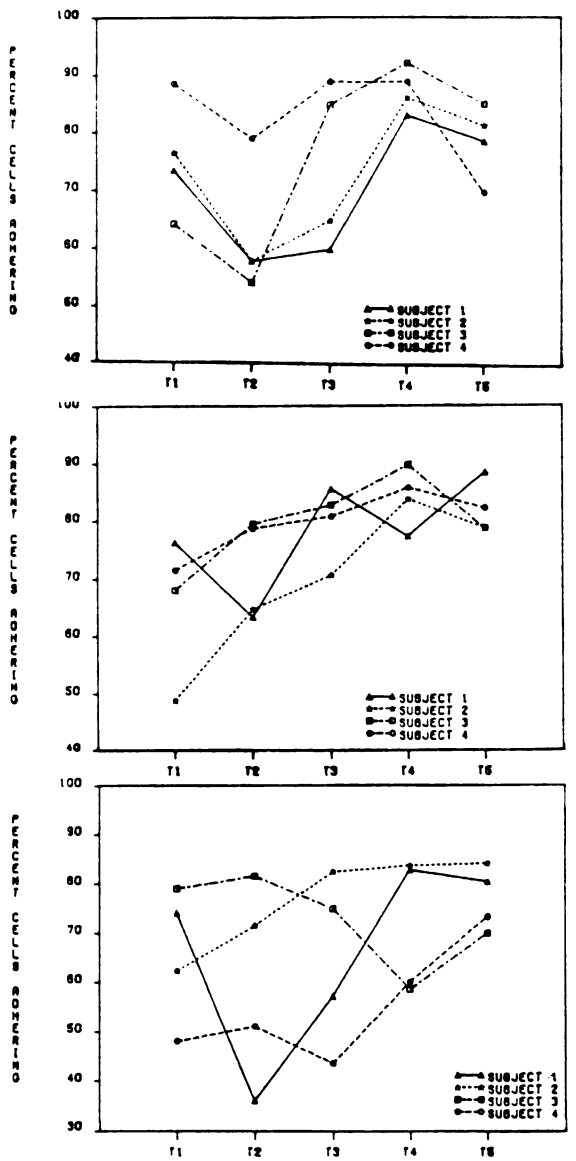
Adherence during baseline for relaxation, imagery, and control groups

Figure 3 demonstrates how marked individual differences in adherence levels during baseline was responsible for the apparent differences between the treatment groups (see Table 2 for individual results). Subjects' baseline adherence was sampled before random assignment to treatment group, thus knowledge of group membership could not have caused the effect. Among the factors that may have contributed to changes in adherence for the relaxation and imagery groups are physical and/or psychological stress of venepuncture, or physical/psychological stress due to experiences immediately preceeding the venepuncture.

Experimental session

Statistical analyses indicated that there were no differences in adherence levels between treatment groups during the experimental condition. The effects of imagery on neutrophil function could not be distinguished from the effects of relaxation, or from the effects of the no-treatment control condition.

Adherence means increased during the second condition for all three groups although the increase was not significant ($f(2,9)=4.16, p=.07$; See Appendix C). An additional group by trial repeated measures ANOVA for the five measurement periods revealed a significant main



LEGEND

T1 Pre-baseline
T2 Post-baseline
T3 Pre-experimental*
T4 Post-experimental*
T5 Follow-up
* pre & post baseline for control subjects

FIGURE 3

Individual results by treatment group

TABLE 2

Individual Results

Adherence for imagery, relaxation, and control groups

(in percent neutrophils adhering)

IMAGERY

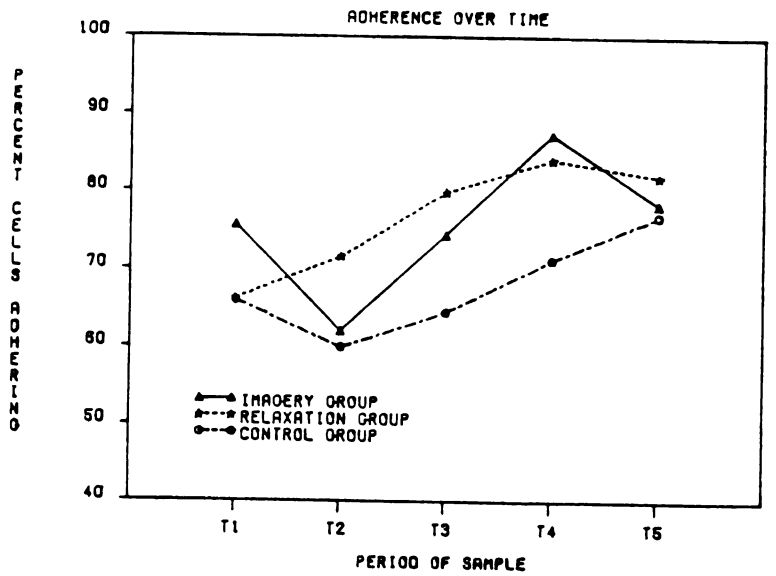
Subject No.	BASELINE			EXPERIMENTAL			FOLLOW-UP
	Pre	Post		Pre	Post		
1	73.48	57.58	15.9	59.67	82.86	-23.2	78.32
2	76.54	57.74	18.8	64.74	85.86	-21.1	81.13
3	64.22	53.95	10.3	84.86	92.71	-7.8	84.83
4	88.54	78.95	9.6	88.82	88.71	.1	69.35

RELAXATION

Subject No.	BASELINE			EXPERIMENTAL			FOLLOW-UP
	Pre	Post		Pre	Post		
1	76.35	63.39	11.0	85.59	77.36	8.2	88.47
2	48.86	64.76	-16.0	70.65	83.79	-13.1	78.69
3	68.08	79.59	-11.5	82.74	89.71	-7.0	78.71
4	71.54	78.76	-7.2	80.79	85.80	-5.0	82.16

CONTROL

Subject No.	SESSION 1			SESSION 2			FOLLOW-UP
	Pre	Post		Pre	Post		
1	74.14	35.90	35.2	57.18	82.75	-25.6	80.35
2	62.37	71.56	-9.2	82.50	83.72	-1.2	84.17
3	79.08	81.61	-11.5	74.88	58.50	16.4	69.85
4	48.15	51.03	-2.8	43.48	60.00	-16.5	73.23



LEGEND

T1 Pre-baseline
T2 Post-baseline
T3 Pre-experimental*
T4 Post-experimental*
T5 Follow-up
* pre & post baseline

FIGURE 4

Adherence over five measures by treatment group

effect for trials ($f(2,4)=5.32, p<.001$; Appendix C; Figure 4). Furthermore, as illustrated in Figure 3 (see also the descriptive statistics in Table 1) individual differences during the sessions decreased over time, suggesting an habituation effect. Subjects who tended to react to venepuncture with decreased adherence may have become less reactive, and all subjects may have experienced less stress with repeated exposure to the experimental situation. Experiments conducted by Ward and her associates (Ward, Mefford, Parker, Chesney, Taylor, Keegan, & Barchas, 1983) corroborate the hypothesis that venepuncture may be associated with stress-induced physiological changes in some subjects.

Evidence for a ceiling effect

A ceiling effect may have obscured differences between treatment effects during the experimental session. As can be seen in Table 1, standard deviations decreased as means increased for trials in the three groups. Cases with the largest increases in adherence had the lowest adherence levels during baseline. Similarly, cases with the highest baseline values increased the least.

The decreased variance suggests a ceiling effect, and makes interpretation of experimental results more difficult. However, because results similar to the two

experimental treatments were found with the control group, it is unlikely that either imagery or relaxation had any effect on neutrophil function.

Summary

Results of the current experiment provided no support for Hypothesis 1: In subjects trained in imagery/relaxation, changes in neutrophil function will be found during an imagery session. Furthermore, imagery and relaxation were not distinguishable, a finding which fails to support Hypothesis 2: Patterns of changes in neutrophil function for subjects trained in imagery/relaxation will differ from patterns found in subjects trained in relaxation alone.

The results of the experiment in general do not support results of the Schneider et. al.(Note 1) experiments. However, as stated above, the adherence measure used in those experiments was not the same as the adherence measure in the current experiments. Furthermore, adherence values were lower and possibly not subject to the same ceiling effect (Note 2).

CHAPTER IV

DISCUSSION

The hypothesis that imagery/relaxation may be associated with changes in neutrophil function cannot be ruled out on the basis of the current research because of the possible ceiling effect, and because of the problems with the adherence measure. However, the current results suggest that neutrophil function may not be affected by imagery/relaxation. The results from the untreated control groups were quite similar to results for both imagery/relaxation and relaxation treatment conditions in the current research. If the relationship between imagery and neutrophil function is robust, one would expect to find a different pattern of findings with an untreated control.

The reasons for the Schneider et. al. (Note 1) findings are unclear. The current results suggest that stress may be an important variable in changes in neutrophil adherence. It is possible that the experimental situation stress level was a co-varying factor with different imagery instructions in the

Schneider et. al. (Note 1) experiments.

In the first of these experiments, in which subjects were asked to image their neutrophils becoming active and emigrating, reduced adherence of neutrophils and reduced white cell count was found. It was hypothesized that the reduced adherence had been caused by the reduction in neutrophil count (increased margination of cells). However, it would seem that reduced adherence might also be caused by stress, perhaps in the form of performance anxiety during the imagery.

The fact that a reduction in adherence was similarly found in another experiment in which subjects imaged their cells becoming less active is problematic for the hypothesis that specific content of imagery is associated with specific activity of the cells. This argument rests on the hypothesis that in the first experiment, in which decreased count and adherence were found, the decreased adherence was caused by the reduced count. This is a hypothesis that requires further experimentation. Fluctuations in white cell count are common, rapid, and seasonal. Count may not be a reliable dependent measure upon which to base hypotheses for this reason.

In the second of the Schneider et. al. (Note 1) experiments, subjects were asked to image their cells becoming more active and not emigrating. Increased neutrophil adherence was found in this experiment,

however, there were other factors co-varying with the instructions, such as a larger subject pool, extensive psychological testing with subjects (Rorschach), and other factors that might have had an impact on the experimental situation stress level.

Future Research

The finding of Increased adherence over the course of the experiment with both imagery and relaxation, demonstrates the need for appropriate controls in future research. If an untreated control had not been added, the results would have suggested that both imagery and relaxation increase neutrophil adherence.

Future research also would do well to explore the patterns of interactions between physiological stress responses and neutrophil function. Results of the current experiment, and perhaps of the Schneider et. al. experiments, might be explained using these notions. Research conducted by Ward, Mefford, Parker, Chesney, Taylor, Keegan, & Barchas (1983) demonstrated alterations in blood plasma levels of catecholamines with physical (venepuncture, hand submersion in ice water) and psychological (anagram solution under pressure) stress. In addition, they found significant individual differences in physiological changes accompanying these stressors.

MacGregors's (1977) research on human neutrophils showed reduced adherence, motility, and response to chemotactic factor with in vitro exposure to epinephrine. Glucocorticoids also alter neutrophil adherence (MacGregor, 1977). Corticosteriods are known to be responsive to physical and psychological stress (Monjan, 1981). A measure of corticosteriod levels would be a necessary addition to further research on the relationship between psychological factors and neutrophil function. Results of such research might show that corticosteriod levels mediate the relationship between neutrophil function and psychological factors.

A final area for future research would be an investigation of personality correlates of reactivity to venepuncture. Some individuals may be more immunologically reactive to stressors than others, and the predictability of reactivity would be important to assess. As with all research in the area, the role of corticosteriods as a mediator in the relationship would have to be an additional focus of investigation.

LIST OF APPENDICES

APPENDIX A

Imagery/relaxation induction procedure

(Adapted from the imagery induction procedure developed by

John M. Schneider, Note 1)

First of all, try to sit in a way that you can be very relaxed and comfortable. Take a moment to settle yourself in the chair so that your arms and your legs, and your back, can be very relaxed and comfortable. Let your body settle down, and begin to imagine that your breathing is slowing down, as you feel more and more comfortable. You might want to pick a spot on the wall or ceiling, and let your eyes go soft. As your eyelids start to feel very heavy, you might want to close your eyes, but you don't have to. Do whatever is comfortable for you.

Imagine that your toes and your feet are starting to feel very warm and heavy. Feel the warmth and heaviness begin to relax your feet and your toes, and then feel it

start to enter your ankles and your calves. Imagine that the warmth and heaviness is relaxing your calves and your knees, as your whole body begins to feel relaxed and warm. Imagine the warmth and heaviness move through your knees into your thighs, relaxing your legs. Your legs now are very still and comfortable.

Imagine that your stomach is starting to let go of the tension you carry there, letting go slowly of the tension, you may feel it replaced with warmth and relaxation. Let the warmth and heaviness relax your stomach, and your back, beginning with your lower back. Let the relaxation move up through your back, relaxing all the muscles, up to your neck, and imagine that your shoulders are starting to feel relaxed. Let go of the tension in your shoulders, and feel your relaxation become still deeper. Your body is feeling quite peaceful now, there is warmth and heaviness in your legs, your stomach, back, and shoulders. Image the warmth and heaviness now in your arms and your hands, right down to your finger tips. Imagine that your neck and face is relaxing, feel the muscles in your face become warm and comfortable.

Your whole body is feeling very warm and heavy, relaxed and comfortable. Breathe in slowly and deeply. Breathe in warmth and heaviness and comfort. Breathe out

any tension. And as your breathe in, say to yourself, I am. and as you breathe out, to yourself, relaxed. I am...relaxed. I am...relaxed. If any thoughts come into your head, just let them go their way. If there are any noises, just let them pass through.

Imagine that you are going on a trip inside your body. You get into an elevator, and descend slowly, deep down into yourself. Imagine that the elevator stops, door opens, and you look out into the amazing world before you. Notice the colors, the shapes, the sounds of this world where you now find yourself. Begin to imagine that you see your neutrophils, in whatever way you like. Imagine that you see them floating in your blood stream. Imagine that they are starting to become active. They begin to change shape, and become very sticky. Imagine them becoming active, changing shape, and becoming sticky. Imagine them start the stick to the walls of the blood vessel and imagine them passing between the cells of the blood vessel wall. Continue to imagine your neutrophils becoming active, changing shape, becoming sticky. Imagine them sticking to the blood vessel wall, and imagine them passing through. Continue to imagine these things on your own for awhile, in whatever way you like. I will let you know when its time to come back to the room. (10 minute interval).

Now begin to imagine that its time to leave this place where your neutrophils are. Let them know that they can go back to doing whatever they were doing before. Get into the elevator, and begin to come back to the room. You can feel the muscles of your body lighten up. Let your eyelids start to get light. Wiggle your toes and your fingers, and you can soon open your eyes and become aware of the room around you. Continue to relax, but awake now, and feeling refreshed.

APPENDIX B

Relaxation induction procedure

(adapted from the imagery induction procedure developed by

John M. Schneider, Note 1)

First of all, try to sit in a way that you can be very relaxed and comfortable. Take a moment to settle yourself in the chair so that your arms and your legs, and your back, can be very relaxed and comfortable. Let your body settle down, and begin to imagine that your breathing is slowing down, as you feel more and more comfortable. You might want to pick a spot on the wall or ceiling, and let your eyes go soft. As your eyelids start to feel very heavy, you might want to close your eyes, but you don't have to. Do whatever is comfortable for you.

Imagine that your toes and your feet are starting to feel very warm and heavy. Feel the warmth and heaviness begin to relax your feet and your toes, and then feel it

start to enter your ankles and your calves. Imagine that the warmth and heaviness is relaxing your calves and your knees, as your whole body begins to feel relaxed and warm. Imagine the warmth and heaviness move through your knees into your thighs, relaxing your legs. Your legs now are very still and comfortable.

Imagine that your stomach is starting to let go of the tension you carry there, letting go slowly of the tension, you may feel it replaced with warmth and relaxation. Let the warmth and heaviness relax your stomach, and your back, beginning with your lower back. Let the relaxation move up through your back, relaxing all the muscles, up to your neck, and imagine that your shoulders are starting to feel relaxed. Let go of the tension in your shoulders, and feel your relaxation become still deeper. Your body is feeling quite peaceful now, there is warmth and heaviness in your legs, your stomach, back, and shoulders. Imagine the warmth and heaviness now in your arms and your hands, right down to your finger tips. Imagine that your neck and face is relaxing, feel the muscles in your face become warm and comfortable.

Your whole body is feeling very warm and heavy, relaxed and comfortable. Breathe in slowly and deeply. Breathe in warmth and heaviness and comfort. Breathe out

any tension. And as your breathe in, say to yourself, I am. and as your breathe out, to yourself, relaxed. I am...relaxed. I am...relaxed. If any thoughts come into your head, just let them go their way. If there are any noises, just let them pass through. Just continue to relax on your own for awhile. Listen to the sound of ocean waves if you like, or do whatever helps you feel very relaxed and comfortable. I will let you know when its time to come back to the room. (15 minute interval).

Now begin to imagine that its time to begin to come back to the room. You can feel the muscles of your body lighten up. Let your eyelids start to get light. Wiggle your toes and your fingers, and you can soon open your eyes and become aware of the room around you. Continue to relax, but awake now, and feeling refreshed.

APPENDIX C

Analysis of Variance Summary Tables

Table 1

Group by Trial ANOVA: Pre to Post-Baseline

	Pre		Post	
Group	Mean	SD	Mean	SD
Imagery	75.69	10.03	62.05	11.39
Relax-ation	66.20	12.05	71.62	8.74
Control	65.93	13.77	60.02	20.59

Source of Variance	Sum of Squares	Df	F	Prob
Group	186.64	2	.38	ns
Error	2203.52	9		
Trial	133.51	1	1.22	ns
Group X Trial	36318.83	1	7.96	.03
Error	984.60	9		

Table 2

Group by trial ANOVA: Pre to post-baseline:

Relaxation and Imagery groups only

	Pre		Post	
Group	Mean	SD	Mean	SD
Imagery	75.69	10.03	62.05	11.39
Relax-ation	66.20	12.05	71.62	8.74

Source of Variance	Sum of Squares	Df	F	Prob
Group	.68	1	.00	ns
Error	108336.98	6		
Trial	6760.95	1	1.48	ns
Group X Trial	36318.83	1	7.96	.03
Error	27373.77	6		

Table 3

Group by Trial ANOVA: Pre to Post Experimental Session

	Pre		Post	
Group	Mean	SD	Mean	SD
Imagery	74.52	14.46	87.37	3.93
Relax-ation	79.94	6.50	84.16	5.15
Control	64.51	17.58	71.24	13.87

Source of Variance	Sum of Squares	Df	F	Prob
Group	994.89	2	2.86	.10
Error	1568.10	9		
Trial	377.78	1	4.16	.07
Group X Trial	78.77	2	.43	ns
Error	816.58	9		

Table 4

Group by trial ANOVA over time (five measures)

Source of Variance	Sum of Squares	Df	F	Prob
Group	972.14	2	2.03	ns
Error	2153.73	9		
Trial	2212.09	4	5.32	.001
Group X Trial	710.76	8	.85	ns

REFERENCE NOTES

REFERENCE NOTES

[1] Schneider, J., Smith, C.W., & Whitcher, S.B. Relationship of mental imagery to white blood cell (neutrophil) function: Experimental studies of normal subjects. Manuscript in preparation.

[2] The measure of adherence used in the Schneider et. al. experiments involved an isolated cell procedure where the number of neutrophils sticking to protein-coated glass was assessed, in contrast to the nylon wool procedure used in the current experiment. Previous research conducted by Smith suggested that the nylon wool technique was conparable to the isolated cell procedure, however in the procedure used by Smith, a volume of 50 mg of nylon wool was packed into a .25 ml volume space. In this experiment, an 80 mg volume of nylon wool was packed into the same volume, as initial trials with the technique indicated that the 50 mg/.25 ml preparation yielded results inconsistent with past research. However, this proved to be an error in technique which was not detected until the experiment had been completed.

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