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Choral Singing, Performance Perception, and Immune System Changes in Salivary Immunoglobulin A and Cortisol

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In a naturalistic pre-post design, samples of saliva were collected from the members of a professional chorale during an early rehearsal ($n = 31$), a late rehearsal ($n = 34$) and a public performance ($n = 32$) of Beethoven's *Missa Solemnis*. As measures of immune system response, mean levels of secretory immunoglobulin A increased significantly, as a proportion of whole protein, 150% during rehearsals and 240% during the performance. Cortisol concentrations decreased significantly an average of 30% during rehearsals and increased 37% during performance. As measured through performance perception rating scales, a group of emotions and other experiential states that singers associated with professional singing were highly predictive of changes in level of secretory immunoglobulin A during the performance condition, but the results for the rehearsal conditions were not significant. The best multiple regression model for performance level of immunoglobulin A ($p < .0015$) included seven emotional, cognitive, and evaluative variables generally associated with choral singing, including levels of mood before and during singing, stress, relaxation, feeling "high," detachment/engagement, and specific satisfaction with the immediate performance.

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T HE purpose of this study was to determine whether choral singing is associated with physiological changes in the immune system. A professional chorale was studied during a period in which they rehearsed and performed Beethoven's complex choral masterwork, *Missa Solemnis*. As described by its practitioners, the successful choral singing experience in public performance is a peak experience during which, in the face of performance challenges and anxiety, the performer has transcendent feelings

of extreme exhilaration, mood changes, relaxation, musical concentration, satisfaction, and diminishing stress. Given that singers perceive choral singing as a highly pleasurable and satisfying experience, as well as an intense physical experience involving considerable exercise of the respiratory system, it was theorized that the activity might stimulate production of immune system proteins, including immunoglobulin A (IgA) and the stress hormone cortisol. It was considered likely, however, that singers' perceived experiences of singing would mediate effects in the physiological system. Our goal was to determine whether singing is associated with changes in levels of IgA and cortisol and to determine the kinds of emotional and other experiences that might be associated with changes in levels of IgA and cortisol.

SECRETORY IMMUNOGLOBULIN A

Although no direct studies of this question have been conducted, behaviors that can be assumed to be part of the singing experience—including positive emotional arousal (Dillon, Minchoff, & Baker, 1985), relaxation (Green & Green, 1987; Green, Green, & Santoro, 1988), positive mood (Stone, Cox, Valdimarsdottir, Jandorf, & Neale, 1987), and pleasurable social events (Miletic, Schiffman, Miletic, & Sattely-Miller, 1996)—have been found to be associated with elevated levels of secretory IgA (S-IgA). Moreover, at least one study found that the elevated levels of S-IgA were relatively long-term and cumulative. Green et al. (1988) reported that a group of subjects who practiced relaxation for 21 days had higher S-IgA concentrations than did subjects in a control group who had practiced for only 1 day. In addition, the long-term-practice group had significantly higher S-IgA levels in saliva samples taken before and after practice on day 22 than in samples obtained previously during the 3-week practice period.

Many studies have shown the converse: that stress has negative effects on S-IgA levels. Martin and Dobbin (1988) found that scores on a daily hassles scale were associated with decreased levels of S-IgA. Academic stress (Jemmot et al., 1983; Kiecolt-Glaser, Garner, Speicher, Penn, Holliday, & Glaser, 1984), incarceration (Borysenko, McClelland, Meyer, & Benson, 1982), high power inhibition in prisoners, and health stress (McClelland, Floor, Davidson, & Saron, 1980) were all associated with decreased levels of S-IgA. Intensive physical activity decreases S-IgA levels (Mackinnon & Hooper, 1994; Tharp & Barnes, 1990). Graham, Bartholomeusz, Tabonpong, & La Brooy (1988) reported that nurses who had more frequent episodes of anxiety had significantly lower mean levels of S-IgA than did nurses who had only occasional episodes of anxiety. However, in the study by Graham et al., it was not possible to determine whether anxiety influenced the secretion of IgA in saliva independently of its effects on salivary flow.

Previous research has suggested that diminished IgA levels might have adverse effects on health. McClelland et al. (1980) found that subjects with

lower S-IgA concentrations reported more frequent illnesses. A number of researchers have correlated stress with upper respiratory tract infections. S-IgA has been used as a marker for the role of mucosal immune defense against infection by respiratory viruses. Depressed levels of S-IgA during stress have been implicated as a potential mechanism by which stress might increase an individual's potential susceptibility to acute respiratory infection (Graham et al., 1988).

CORTISOL

On the other hand, pleasant emotional states induced by "positive mood" film segments (Hubert & de Jong-Meyer, 1990) were associated with decreased cortisol levels. However, Green and Green (1987) did not find that relaxation reduced cortisol level in saliva. Because of the paucity of studies, the question of whether induction of immunoenhancement decreases cortisol levels in saliva remains unanswered.

The evidence is much more substantial for the effects of stress on cortisol levels. Increased cortisol levels have been related to a wide range of stressful life experiences and experimental conditions, including dental exams (Benjamins, Asscheman, & Schuurs, 1992), parachute jumping (Cook, Read, Walker, Harris, & Ria-Fahmy, 1992), separation effects in infants (Larson, Gunnar, & Hertsgaard, 1991), attention tests with mild electric shock (Muller, Budde, & Netter, 1992), and being held hostage in Iran for 444 days (Rahe, 1990).

PERFORMANCE PERCEPTIONS MEDIATING SINGING AND IMMUNE FUNCTIONS

Martin and Dobbin (1988) found that subjects who have relatively low scores on sense-of-humor scales have a stronger negative relationship between daily hassles and S-IgA levels than do other subjects. Dillon et al. (1985) reported that IgA levels increased significantly after subjects watched a humorous film and that scores on a Coping Humor Questionnaire were correlated with IgA concentrations averaged over several conditions. In these studies, a good sense of humor, apparently, enabled subjects to cope better with self-reported daily hassles, and the results suggested that physiological changes occur in response to humorous experiences. However, when Dillon et al. looked at the changes in subjects' IgA levels from before to after the subjects watched the humorous film, scores on the humor questionnaire were significantly negatively correlated with changes in IgA. The researchers concluded that subjects who used sense of humor as a coping device may already have been closer to a hypothetical ceiling to changes in IgA as a function of positive emotional experience.

The effects of positive psychological states on S-IgA and cortisol levels are mediated by levels of practice, cognitive variables, and social support.

For example, Green et al. (1988) found that the increase in the rate of S-IgA secretion was greater in subjects who had practiced relaxation once a day for 3 weeks than it was in control subjects who were practicing relaxation for the first time. This difference suggests that the length of time that singers had engaged in choral singing might influence their singing experiences and associated immune functions. Kirschbaum and Hellhammer (1994) reported (unpublished data) that after a bicycle ergometry stimulation, which normally would have increased subjects' levels of cortisol, male subjects were found to have decreased levels of cortisol when they were receiving social support from their girlfriends (however, the opposite was true for female subjects.) Comparable to the effects of humor and relaxation on S-IgA levels, therefore, effective coping abilities during singing may lead to stronger immunoenhancement. In choral singing, social support is evidently very much a part of the experience.

A PRELIMINARY MODEL OF CHORAL SINGING AND THE IMMUNE SYSTEM: COPING WITH PERFORMANCE ANXIETY

Although professional choral singing is undoubtedly the source of a range of positive emotional experiences and perceived benefits for its practitioners, it is nonetheless a naturally anxiety producing activity, particularly before and during public performances. Every performance is a test of a chorale's excellence in interpreting and vocalizing a particular work of musical literature. The results of this test are as much determined by singers for themselves as by responses of the director, the audience, and ultimately critics. During choral singing, performers cope more or less successfully with the stressors of performance. We propose a model, therefore, that successful adaptations are likely to be accompanied by characteristic subjective states, such as satisfaction with performance, feelings of positive emotional arousal, relaxation, and group fellowship. However, unsuccessful adaptations, that is, poor performances, would probably allow feelings of performance anxiety and stress to persist.

Are there mediating variables in the perception of choral singing, therefore, that are comparable to the function of humor in coping with stress? In singing, there are several natural means of coping with and mastering difficult pieces. During performance, singers need to use highly focused concentration on the music. The musical literature of professional chorales is commonly highlighted by works that are deeply spiritual and emotionally profound. Additionally, in a chorus, a large group shares the problem of delivering a high level of performance. These problem-solving activities and feelings associated with high musical involvement and meaningfulness may help singers to overcome the natural stress and anxiety of performance. Comparable perhaps to a sense of humor, a "sense of singing" consists

theoretically of abilities to tap into, create, and express the positive emotions and other experiences that are potentially derived from choral singing. Do singers' perceptions of positive emotional subjective states and satisfaction with their performance modify the effects of singing on immunoenhancement? Previous studies directly relevant to the experience of singing have shown that relaxation and positive mood, in particular, are associated with immunoenhancement. Therefore, would the strength of singers' agreements that such states as relaxation and positive mood were part of their experience of singing in general and/or their agreements that particular singing sessions were satisfying and enjoyable tend to modify immunoenhancement in a positive direction, that is, be associated with increases in IgA concentration and decreases in cortisol level? It was expected that these positive emotional states would help singers overcome the natural anxiety associated with public performances and with rehearsals to a lesser extent.

IMMUNOGLOBULINS IN SALIVA

Saliva is a complex mixture of secretions from several glands including rhe parotid, submandibular, and sublingual glands. These glands are the source of S-IgA in the upper gastrointestinal and respiratory tracts. The concentration of S-IgA is influenced by the volume of salivary flow. Flow is determined by a variety of factors including food ingestion, drugs, smoking, body positioning, physical exercise, and degree of hydration (Dawes, 1993; Kiecolt-Glaser & Glaser, 1988). Mackinnon and Hooper (1994) reported that the protective effect of S-IgA in the upper gastrointestinal and respiratory tracts is dependent on both absolute S-IgA concentration and on salivary flow. Stress tends to reduce flow rates, and this reduced flow may account for higher concentrations of S-IgA found under such conditions and hence provide false indicators of immunoenhancement (Carlson, 1986). Nurses who reported more frequent episodes of anxiety had significantly lower mean secretion rates of S-IgA than did nurses who reported only occasional episodes of anxiety. However, it was not possible to determine whether anxiety influenced S-IgA secretion in saliva independently of its effects on salivary flow, which is controlled by the autonomic system. Miletic et al. (1996) found that both saliva flow and S-IgA concentrations were significantly lower in the "elderly" (60–80 years old) than in the "young" (20–30 years old). Therefore, measurements of S-IgA and cortisol levels must be controlled for salivary flow. In the present study, salivary flow was controlled for by assessing concentrations of target proteins in proportion to whole protein.

Synthesis of antibodies or immunogloblulins is one of the terminal events in the normal immune response. The S-IgA isotype is one of the five differ-

ent classes of antibodies in the human immune system and is the dominant antibody in the secretory, mucosal immune system, where, at the various ports of entrance to the body, it functions as a first line of defense against microbial infections (Miletic et al., 1996). In particular, S-IgA is the body's first line of defense against respiratory illness (Brandtzaeg, Fjellenger, & Gjeroldsen, 1970). S-IgA is found in saliva, intestinal secretions, bronchoalveolar lavage fluids, urine, tears, and other mucosal fluids (Carayannopoulos & Capra, 1993). Analysis of the immune response in saliva can provide an index of the function of the entire mucosal immune system (Mestecky, 1993). S-IgA synthesis is T-cell dependent, that is, B cells need T cells to switch from producing IgM antibodies to IgA antibodies (Snapper & Finkelman, 1993).

Miletic et al. (1996) offered a number of persuasive reasons for the use of salivary S-IgA levels to evaluate immune status: (1) saliva is easily retrieved and can be collected several times per day, even in young populations; (2) because the synthesis rate of S-IgA is high and its half-life is short, changes in S-IgA concentration can be detected in the short term; (3) changes in synthesis can be correlated directly to T and B cell activation; and (4), because there is cooperation between distant compartments in the mucosal immune system, the S-IgA concentrations in saliva may be generalized to the entire mucosal system.

CORTISOL IN SALIVA

Because of the noninvasiveness and laboratory independence of sampling, salivary cortisol levels also can be measured with great frequency in a variety of settings. Moreover, unlike S-IgA, cortisol levels are unaffected by saliva flow rate (Kirschbaum & Hellhammer, 1994). Salivary cortisol concentration is a good indicator of immune system functioning because salivary cortisol is a potent form of unbound cortisol. The salivary glands prevent protein-bound molecules from entering saliva. Only unbound cortisol has been conceptualized to elicit glucocorticoid effects.

Methods

The objective of the study was to determine if choral singing is associated with immunoenhancement. The principal independent variable was choral singing, and the dependent variables were measures of S-IgA and cortisol concentrations in saliva. A naturalistic study was conducted by using pre-singing and post-singing measures of S-IgA and cortisol levels during two rehearsals and one performance that had been previously scheduled by the professional chorale. In effect, the rehearsals and performance served as natural treatment conditions. However, no control group was used in the research design. One could argue that no control group was needed because the concentration of a protein such as IgA is not expected to change during singing, or, if anything, it would change negatively, be-

cause strenuous exercise had previously been determined to correlate negatively with IgA concentration (Mackinnon & Hooper, 1994; Tharp & Barnes, 1990). Nevertheless, it would be more rigorous to use a control group design, if only to rule out unexpected natural diurnal variations in IgA and cortisol concentrations. Although diurnal rhythms of cortisol level in humans are well known, with low points occurring in early evening, no published data were available on diurnal rhythms of IgA concentration when this study was done.

As measured through rating scales, the singers' perceptions of choral singing under different conditions were expected to modify the relation between choral singing and pre-post changes in IgA and cortisol levels.

SUBJECTS

The singers of the Pacific Chorale, a professional performing group ($N = 180$), constituted the initial pool from which a sample of singers was selected. Singers who exhibited any of the following characteristics, which have been associated with decreased immune system functioning (Kiecolt-Glaser & Glaser, 1988), were excluded from the pool before selection of the final sample: consumption of more than 10 alcoholic drinks per week; smoking more than a pack of cigarettes or five cigars per week; current illness or having had an illness in the past month; having had weight changes (5% or more of total body weight) in the past 4 months; or experiencing insomnia two or more nights per week. By these criteria, 28 singers were excluded from the pool. From the study pool of 152 singers, 41 were randomly selected, including 23 female singers (56.1%) and 18 male singers (43.9%). The population had a mean age of 46.4 years (range, 25–62 years). They had been singing in choral groups for an average of 36.6 years (range, 10–55 years). Clearly, many of the singers had been involved with singing groups since childhood. As a result of absences, insufficient saliva samples, and other problems, the following sample groups provided data suitable for analysis: first rehearsal ($n = 31$), second rehearsal ($n = 34$), and performance ($n = 32$).

PROCEDURE

Saliva samples were collected immediately before (7 PM) and after (9:30 PM) an early rehearsal and a late rehearsal and before (8 PM) and after (9:30 PM) a stage performance of Beethoven's choral masterwork, the *Missa Solemnis*. A total of six samples were collected.

SALIVA COLLECTION AND LABORATORY TESTS

A convenient sampling device, "Salivette" (Sarstedt, Inc., Rommelsdorf, Germany) was used for collecting saliva. The Salivette consists of a small cotton swab that fits inside a standard centrifugation tube. Subjects chew gently on the swab, stimulating saliva flow that provides adequate material in 30 to 60 s. The Salivette also facilitates pipetting of the sample because the debris is separated from the clear watery saliva supernatant. The Salivettes have been described as the most convenient device for self-administered saliva collection (Kirschbaum & Hellhammer, 1994).

A problem in using saliva for measuring concentrations of protein is how to overcome the well-known inverse relationship between the amount of saliva produced in a given period (flow rate) and the concentration of S-IgA protein (Brandtzaeg, 1971). Our method controlled for flow rate by also measuring the total amount of protein in the saliva. Thus changes in concentrations of S-IgA or cortisol are expressed as proportions of total protein. Although total protein levels may also increase after singing as a result of dehydration (diminished flow rate) of the saliva, nevertheless, we were able to measure whether or not the increase (decrease) in the levels of the target proteins was greater (less) than that of the total protein.

To demonstrate that IgA and cortisol levels increased as a result of choral singing, therefore, the effects of dehydration were taken into account by using the following equation to calculate mean change as a proportion of total protein[1]:

$$\frac{\dfrac{\text{Post-Singing IgA}}{\text{Post-Singing Protein}} - \dfrac{\text{Pre-Singing IgA}}{\text{Pre-Singing Protein}}}{\dfrac{\text{Pre-Singing IgA}}{\text{Pre-Singing Protein}}} \tag{1}$$

In this way, changes in pre-singing and post-singing IgA levels were adjusted for changes in whole protein levels. The changes in IgA are expressed as mean changes as a proportion of total protein. A similar equation was used to calculate mean change in cortisol level as a proportion of total protein:

$$\frac{\dfrac{\text{Post-Singing Cortisol}}{\text{Post-Singing Protein}} - \dfrac{\text{Pre-Singing Cortisol}}{\text{Pre-Singing Protein}}}{\dfrac{\text{Pre-Singing Cortisol}}{\text{Pre-Singing Protein}}} \tag{2}$$

All IgA and cortisol concentrations were converted to milligrams per milliliter.

Laboratory analyses of the proteins were conducted according to standard procedures recommended by manufacturers of the tests. IgA was measured quantitatively through the Kallestad Endoplate single radial immunodiffusion test.[2] Cortisol was measured quantitatively through the Coat-A-Count radioimmunoassay test.[3] Whole protein was measured quantitatively by the Lowry Biorad test.[4]

RATING SCALES OF PERFORMANCE PERCEPTIONS ASSOCIATED WITH CHORAL SINGING

A "Singers Emotional Experiences Scale" (SEES) was devised by the authors and administered to the sample. The SEES consists of 28 Likert-scale questions about levels of agree-

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1. With respect to equations 1 and 2, if a is a pre-singing proportion and b is a post-singing proportion, and if $x = (b - a)/a$, this does not mean that the mean of the proportionate changes, $\bar{x} = (\bar{b} - \bar{a})/\bar{a}$.

2. Test plates contain a layer of gel embedded with anti-IgA. Saliva samples are loaded into the wells and incubated at room temperature for 48 hours. The IgA diffuses from the well into the gel. The resulting diameter is read with a microscope and converted by a calibration curve to weight (mg/dL). The normal range is 3–25 mg/dL.

3. The Coat-a-Count commercial radioimmunoassay kit was designed by the manufacturer for determining cortisol in saliva and was not modified by the investigators. All assay procedures were conducted following manufacturer instructions. The antibody is immobilized to the wall of a polypropylene tube. The saliva sample is added to the tube. Decanting the supernatant suffices to terminate the competition and to isolate the antibody-bound fraction of the radiolabeled cortisol. Counting the tube in a gamma counter then yields a number, which converts by way of a calibration curve to a measure of the cortisol present in the sample (mg/dL). The normal range is 1–50 mg/dL.

4. In the procedure, protein reagent is placed in a well. The saliva sample is introduced into the well. The chemical reaction develops color. The color is read through a photospectrometer, which measures color density. A calibration curve is used to convert the reading to weight (mg/mL). The normal range is 1–3 mg/mL.

ment with various emotions and other experiences typically experienced before, during, or after singing in rehearsals and performances. How do singers perceive or conceptualize the positive (or negative) emotions and benefits (or costs) they experience in singing? Our goal was to model experiential states, emotions, and evaluations that singers shared and that might mediate predicted physiological changes. Although the SEES is a new, unproven scale, some informal evidence supports the construct validity of the items used in the test. Several characteristic experiences associated with choral singing were frequently mentioned in informal pilot conversations with singers who were not in the sample and subsequently confirmed through the SEES. Although we believe that the SEES may adequately capture the content of the salient emotions associated with singing, it is nonetheless open to question whether a verbal scale is sufficiently sensitive to discriminate the kinds of nuances of feelings that are part and parcel of this complex experience.

In summary, the SEES consisted of statements that referred to *typical* experiences connected with rehearsals or performances before, during, and after singing. Subjects were asked whether they agreed very much, agreed, neither agreed nor disagreed, disagreed, or disagreed very much with each item.

A 10-item Likert-scale "Self-Report Form" (SRF) was devised by the authors and administered after each rehearsal and performance to measure personal perceptions and evaluations of experiences associated with singing on *particular* days. The SRF consisted of 10 items, including both positive emotional experiences (e.g., "I felt that today's rehearsal [or performance] was exciting") and negative emotional experiences (e.g., "I was frustrated with my performance today"). Our objective was to determine whether singers evaluated the rehearsals differently from each other and from the performance. As part of our model of choral singing, it was assumed that singing during performances was associated with both more positive emotions and more negative emotions than rehearsals. Thus, we expected that singers would rate the performances as higher in "satisfied," "exciting," and "enjoyed" categories and lower in the "frustrated" category when compared with rehearsals. We also expected that performances might be higher in negative emotions than the rehearsals and so would be rated higher in the "stress" and "anxious" categories than would rehearsals.

In summary, the following SRF evaluation measures were used to compare rehearsals and performances and to predict changes in immune system indicators: stress, satisfaction, anxiety, frustration, excitement, and enjoyment.

Both the SEES and SRF instruments examined feelings of stress and anxiety during singing.

HYPOTHESES

Hypothesis 1. It is hypothesized that after rehearsals and performances, S-IgA levels will increase as a proportion of total protein as measured by pre-post differences.

Hypothesis 2. It is hypothesized that after rehearsals and performances, cortisol levels will decrease as a proportion of total protein as measured by pre-post differences.

Hypothesis 3. It is hypothesized that after rehearsals and performances, the effects of singing on pre-post differences in IgA and cortisol levels will be mediated by selected positive and negative perceptions, emotions, and other experiences associated with singing, as measured by the SEES and SRF.

Results

IMMUNOGLOBULIN A

As can be seen in Table 1, concentrations of S-IgA increased in all three conditions, thereby supporting Hypothesis 1. The mean increases in IgA after rehearsals were approximately 1.5 times. The ratio of IgA to whole

TABLE 1
Changes in IgA After Rehearsals and Performances

Condition	n	Mean	SD	Minimum	Maximum
First rehearsal	31	1.49	3.22	−1.000	13.477
Second rehearsal	34	1.58	2.48	−1.000	10.075
Performance	32	2.41	3.37	−0.558	12.235

These changes are based on values obtained with the equation used to calculate IgA in this study, which measures the concentration of IgA as a proportion of whole protein.

protein increased from a mean of 0.04 (SD = 0.042, range = 0–0.18) to 0.06 (SD = 0.048, range = 0–0.21) after the first rehearsal; and from a mean of 0.05 (SD = 0.044, range = 0–0.18) to 0.10 (SD = 0.08, range = 0–0.31) after the second rehearsal. A paired t test was performed on whether the mean change from presinging to postsinging was significantly different from zero for the first and second rehearsals. Both upward changes were significant (first rehearsal: t = 2.984, df =50, p < .004; second rehearsal: t = 4.807, df = 50, p < .0001).

The mean increase after performances was greater than rehearsals, about 2.4 times. The ratio of IgA to whole protein increased from a mean of 0.036 (SD = 0.03, range = 0–0.10) before the performance to 0.073 (SD = 0.049, range = 0–0.197) after the performance. A paired t test was performed on whether the mean change from pre-singing to post-singing was significantly different from zero for the performance. The upward change was highly significant (t = 5.873, df = 50, p < .0001).

Thus, it appears that singing for $2\frac{1}{2}$ hours during rehearsals and $1\frac{1}{2}$ hours during performances was associated with strong increases in IgA. Moreover, the performance condition was associated with higher production of IgA than the rehearsals for the groups tested. In the upper ranges of production, eight singers (25% of the total population sampled) in the performance condition increased their IgA by more than 3.5 times, whereas only three singers (<10% of total) in the first rehearsal and four (12.5% of total) in the second rehearsal increased their IgA by more than 3.5 times. In the lower ranges, 10 singers in the first rehearsal (almost 33.3% of the total population) and 6 singers in the second rehearsal (about 16.6%) showed decreased levels of IgA after singing. In the performance condition, however, only three singers (<10%) had negative changes in IgA. Thus, more than 90% of singers in the performance condition exhibited increases in IgA, and 25% increased IgA to more than 350%.

When an F test was used, none of the independent demographic variables (sex, age, and number of years singing) achieved statistical significance in predicting rehearsal or performance IgA. Similarly, within-subjects' correlations of IgA change across rehearsal and performance conditions

were also nonsignificant. Thus, individual changes in IgA during rehearsals were not significantly correlated with changes during the performance.

CORTISOL

As shown in Table 2, an approximately 0.3 times average decrease in cortisol was associated with the rehearsals, whereas a 0.37 times increase was associated with the performance condition. Therefore, the results support Hypothesis 2 for rehearsals in that cortisol levels did decrease significantly, but that hypothesis is rejected for performances. Apparently, performance stress or anxiety may have remained at higher mean levels during performances than during rehearsals. The ratio of cortisol to whole protein decreased from a mean of 0.00060 (SD = 0.000064, range = 0–0.00316) to 0.00030 (SD = 0.00031, range = 0–.00157) after the first rehearsal and from a mean of 0.000657 (SD = 0.00084, range = 0–0.0047) to 0.00027 (SD = 0.00022, range = 0–0.00085) after the second rehearsal. The ratio of cortisol to whole protein in the performance condition increased from a mean of 0.000451 (SD = 0.00045, range = 0.000025–0.0022) before the performance to 0.000494 (SD = 0.00045, range = 0–0.0021) after the performance.

A paired t test was used to determine whether the mean cortisol change from pre-singing to post-singing was significantly different from zero for the first and second rehearsals. Both downward changes were significant: first rehearsal (t = –2.71, df = 36, p < .01); second rehearsal (t = –2.82, df = 37, p < .007). A paired t test was performed on whether the mean change in cortisol from pre-singing to post-singing was significantly different from zero for the performance. The upward change was significant (t = 2.282, df = 38, p < .028).

Results of an F test indicated that none of the independent demographic variables (sex, age, and number of years singing) achieved statistical significance in predicting rehearsal or performance cortisol. Similarly, within-subjects' correlations of cortisol change across rehearsal and performance conditions were also nonsignificant. Thus, individual changes in cortisol

TABLE 2
Changes in Cortisol After Rehearsals and Performances

Condition	n	Mean	SD	Minimum	Maximum
First rehearsal	34	−0.346	0.681	−1.000	2.339
Second rehearsal	36	−0.285	0.665	−1.000	2.024
Performance	37	0.372	1.089	−1.000	4.440

These changes are based on values obtained with the equation used to calculate cortisol in this study, which measures the concentration of cortisol as a proportion of whole protein.

during rehearsals were not significantly correlated with changes during the performance.

<div align="center">SINGERS EMOTIONAL EXPERIENCE SCALE (SEES)</div>

In informal conversations before rehearsals or performance, many singers had communicated strong feelings of positive anticipation but also anxiety about their task. Many singers spoke of experiencing strong emotions during singing, although depending on the particular work, these might involve various moods ranging from feeling high or exultant to feelings of release or relaxation. Satisfaction with one's performance was regarded as an important factor in affording positive feelings and the reduction of anxiety. Many singers also spoke of a sense of exhilaration or an afterglow that accompanied successful performances. Group fellowship and spirituality were discussed as important benefits of membership in a chorale. The psychological experiences described by singers included such states as "feeling a kind of high," "relaxation," "changes in mood," and "stress."

We first examined the distributions of the individual items on the SEES. In general, the singers agreed with items that referred to positive experiences when singing, such as "singing has made me a happier person" and disagreed with items that referred to negative experiences, such as "singing lowers my mood." The following 13 positive emotion items of 19 total positive emotion items were agreed with very much or agreed with by 50% or more of the sample:

> Singing is very important to me. (92.5%)
> Singing has made me a happier person. (80.5%)
> I feel exhilarated after a performance. (70.7%)
> Singing has contributed to my personal well-being. (68.3%)
> Singing is usually a release. (65.9%)
> Singing gives me identity as an artist. (65.9%)
> I feel exhilarated after singing. (65.9%)
> Singing gives me a kind of high. (63.4%)
> I feel something deep inside me when singing. (58.5%)
> I feel strong emotions when singing. (56.1%)
> I feel singing has made me healthier. (56.1%)
> Singing gives me a strong spirirual feeling. (51.2%)
> After singing I usually feel energized. (51.2%)

Moreover, the following 3 negative emotion items of a total of 9 negative emotion items were disagreed with and disagreed with very much by 50% or more of the singers:

> Singing generally lowers my mood. (57.5% said No)
> I don't usually feel strong emotion when singing. (56.1%)
> I don't usually get a spiritual feeling when singing. (53.7%)

In conclusion, this sample group rated choral singing in extremely positive terms. However, the SEES did not discriminate between singing in rehearsals versus singing in performance. Were there differences in ratings when singers evaluated specific rehearsals as compared with how they rated specific performances?

SELF-REPORT FORM: REHEARSALS VS. PERFORMANCE

Although significance tests were eventually run on all measures, the following four measures of the SRF were discarded as not likely to be useful in analyses: such variables as "the director was very demanding of the chorale today," "the director wasn't pleased with me today," and "the director was critical of me today" were more likely to be applicable during rehearsals but not during performances; the variable, "I had a stressful day before the rehearsal (or performance)" was collected to predict immune system indicators (it proved to be nonsignificant), not to distinguish between evaluations of rehearsals and performance.

The positive emotions "satisfied," "exciting," and "enjoyed" were associated significantly more with performances than with rehearsals (Table 3). Singers rated "satisfied" significantly more associated with the performance than with the first rehearsal ($t = 2.901$, $df = 32$, $p < .007$), but the difference between the second rehearsal and the performance was not significant. The singers rated "exciting" significantly more associated with performance than with the first rehearsal ($t = 6.96$, $df = 31$, $p < .0001$) and more than with the second rehearsal ($t = 7.565$, $df = 31$, $p < .0001$). Singers rated "enjoyed" significantly more associated with performances than with the first rehearsal ($t = 3.917$, $p < .0005$) and more than with the second rehearsal ($t = 3.091$, $p < .004$). Thus, positive emotions were consistently rated higher after performances.

Although we had conjectured that rehearsals would be more frustrating to singers, because the material was difficult and many were learning the piece for the first time, it was believed that the performance would be less frustrating because the material had been mastered to a greater degree. A test of the differences between the rehearsals and performance on this variable confirmed this impression. The performance was rated significantly less frustrating than either the first rehearsal ($t = -3.7731$, $df = 33$, $p < .0006$) or the second rehearsal ($t = -3.125$, $df = 34$, $p < .004$).

Were the performances also associated with negative emotions, as we suspected? In this case, the results were mixed. No significant differences were detected between performances and rehearsals on the variable, "I felt that today's rehearsal (performance) was stressful." The performance, however, was rated as significantly more "anxious" than the first rehearsal ($t = 4.143$, $df = 32$, $p < .0002$) and the second rehearsal ($t = 4.659$, $df = 34$, $p < .0001$).

TABLE 3
Self-Report Form: Differences Between First Rehearsal (F), Second
Rehearsal (S), and Performance (P) Conditions

Variable	Mean	SD	t	Prob > t
Stressful FS	−0.088	1.334	−0.386	.702
Stressful FP	−0.272	1.206	−1.299	.203
Stressful SP	0.086	1.147	0.442	.661
Satisfied FS	0.441	1.160	2.218	.034*
Satisfied FP	0.697	1.380	2.901	.007**
Satisfied SP	0.057	0.968	0.349	.729
Director Demanding FS	0.235	1.257	1.092	.283
Director Demanding FP	0.353	1.152	1.787	.083
Director Demanding SP	0.167	1.028	0.973	.337
Exciting FS	0.344	0.937	2.075	.046*
Exciting FP	1.250	1.017	6.960	.0001***
Exciting SP	0.853	0.657	7.565	.0001***
Anxious FS	0.206	0.845	1.421	.165
Anxious FP	0.879	1.219	4.143	.0002***
Anxious SP	0.857	1.089	4.659	.0001***
Director not pleased FS	−0.303	0.984	−1.769	.086
Director not pleased FP	−0.576	0.902	−3.665	.0009***
Director not pleased SP	−0. 206	0.770	1.559	.129
Frustrated FS	−0.242	1.299	−1.071	.292
Frustrated FP	−0.735	1.136	−3.773	.0006***
Frustrated SP	−0.486	0.919	−3.125	.004***
Stressful day FS	0.121	1.576	0.442	.662
Stressful day FP	−0.212	1.746	−0.698	.490
Stressful day SP	−0.676	1.552	−2.542	.016**
Director critical FS	−0.219	0.751	−1.648	.109
Director critical FP	−0.353	0.812	−2.534	.016**
Director critical SP	−0.176	0.758	−1.358	.184
Enjoyed FS	0.250	1.016	1.391	.174
Enjoyed FP	0.781	1.128	3.917	.0005***
Enjoyed SP	0.438	0.801	3.091	.0042***

FS = difference between first rehearsal and second rehearsal, FP = difference between first rehearsal and performance, SP = difference between second rehearsal and performance. $*p < .05$, $**p < .01$, $***p < .001$.

Thus, the results showed, in general, that performances were associated with both positive and negative emotions more than were the rehearsals. To refine this model further, we examined singers' responses on both the SEES and SRF rating scales in relation to changes in IgA.

ASSOCIATIONS BETWEEN PERFORMANCE PERCEPTIONS, CORTISOL, AND IGA

In general, the pre- and post- cortisol concentrations obtained were far below the normal range of 1–50 mg/dL. Cortisol concentrations were extremely low, ranging from undetectable to only 0.43 mg/dL. This result suggests that the stress levels associated with either the anticipation or per-

formance of singing were not sufficiently strong to stimulate normal levels of cortisol production. To account for the low baseline level of cortisol, we suggest that the long-term effects of singing may have diminished the potential for cortisol production during this activity, or other factors that were not detected by the research design may have been operative. For example, the naturalistic approach required collection of saliva samples at or near the lowest point of diurnal cortisol production levels. Because cortisol levels were below the normal range, it was decided to exclude these data from further analyses and to examine only changes in IgA concentration in relation to the other measures.

In the following analysis, we used the performance perceptions from SEES and from the SRF to predict change in IgA concentration. A stepwise multiple regression analysis was performed on selected variables from the SEES and SRF instruments for the dependent variable IgA. There were no significant models that predicted IgA changes in either rehearsal when variables obtained with the psychological instruments were used.

The remainder of our analysis is devoted to developing a model for predicting performance IgA. Variables were selected according to several criteria. For example, "relaxation" was selected because previous research had demonstrated a relationship of this psychological state to change in IgA level. "Stress" was selected on the basis of our model of singing as a stressful task to which adaptive coping responses operated on both a psychological and physiological level. Other variables from the SEES were found to be correlated individually with IgA level, including "relaxed," "high," "spiritual," and "release." Other significantly correlated variables with S-IgA were taken from the SRF, including satisfied and frustrated. We experimented statistically with the optimal number of items to run through the multiple regressions. The best model consisted of six variables from the SEES and one variable from the SRF. In the multiple regression model for the dependent variable performance IgA change, the results showed that the seven-variable model explained 0.633 (R^2) of the variance, $N = 29$, $F = 5.183$, $p < .0015$ (Table 4). The findings, therefore, support Hypothesis 3, that emotions and experiences associated with singing mediate the physiological effects of singing. Beginning with the strongest predictor, the following variables in order of strength were contained in the performance IgA change model:

> I feel relaxed when singing. (SEES)
> Singing gives me a kind of high. (SEES)
> Before singing, my mood usually rises. (SEES)
> I usually feel detached when singing. (SEES)
> I was satisfied with my performance. (SRF)
> I usually feel stressed during performances. (SEES)
> Singing generally lowers mood. (SEES)

TABLE 4
Stepwise (Forward) Multiple Regression for Dependent Variable
Performance IgA

Variable	Step	Partial R^2	Model R^2	F	Prob > F
Relaxed	1	0.2116	0.2116	7.2457	.0121
High	2	0.0778	0.2893	2.8451	.1036
Mood rises before	3	0.1126	0.4020	4.7084	.0397
Detached	4	0.0749	0.4769	3.4377	.0761
Satisfied	5	0.0696	0.5465	3.5316	.0729
Stressed	6	0.0370	0.5844	2.0051	.1708
Lowers mood	7	0.0490	0.6334	2.8049	.1088

F value = 5.183, $p < .0015$
$R^2 = 0.6334$
$n = 29$

The model suggests that singing is a complex perceptual experience composed of emotional, cognitive, and evaluative states. We need not assume that these states occur simultaneously, but in some sense they may be concurrent at various moments. The model supports the view that performance stress is part of the equation of the singing experience. But adaptive responses are present as well. The model indicates that relaxation, for example, is an important feeling during singing. Also present in the model is a level of detachment, which is probably better described as its opposite, engagement (frequently described, qualitatively, as focused concentration on the music). The model contains several positive emotional states and moods that indicate enjoyment of the experience. These include both feelings of strong arousal ("high") as well as quiet emotions (relaxation). As part of the model, the anticipation of singing is described as accompanied by changes in mood level, and choral singing is associated with changes of mood. The model also contains an evaluative state, satisfaction with performance.

Although the multiple regression model tells us simply that certain factors were collectively part and parcel of the singing experience that was associated with IgA change, the variables did not necessarily individually predict IgA. However, some variables—high, relaxed, and satisfied—were significantly correlated with IgA. To determine the probable direction of each variable in the model, frequencies, Pearson correlations with IgA, and qualitative responses about feelings before, during, and after singing were inspected for each variable. The following discussion is useful in understanding the probable direction each variable assumed for singers in the upper range of the continuum of S-IgA change.

The results showed that singing may have an element of *stress* for some singers (31.8% agreed that singing was stressful or were noncommittal). With respect to the performance, 43.6% of singers reported feeling anx-

ious before singing, but 38% disagreed or disagreed very much that they felt unstressed by singing. However, as an individual variable, stress was not significantly correlated with performance IgA. Therefore, although we can say that the experience of stress is a factor in singing experience that is associated with IgA change, we cannot assume that *lowered stress* is associated with higher IgA, for example. In the multiple regression model, stress is an important factor in that it interacts with other variables in the model to collectively predict performance IgA. On the other hand, singing is probably positively associated with a state of *relaxation* (82.9% agreed very much or agreed). Moreover, relaxation as an individual variable correlated significantly in a positive direction with performance IgA ($r = .483$, $p < .005$). We may tentatively conclude that singing experiences that are associated with high IgA production probably are best described as highly relaxing. Strong positive feelings, *a kind of high*, were also convincingly a part of the singing experience that was positively associated with high IgA change. It is likely that there was a *positive association* between the strength of this feeling and IgA change. The individual correlation of feeling high with IgA was significant in a positive direction ($r = .383$, $p < .03$).

It is highly unlikely, however, that singers feel *detached* during singing (78% disagreed or disagreed very much). In their qualitative descriptions of singing, 43.6% described singing as "focused concentration on the music," which we take to mean that they felt "engaged" during singing. Singers may have typically experienced *changing moods* in anticipation of singing. But, they also overwhelmingly rejected the idea that singing lowers mood (87.5% disagreed or disagreed very much). The level of *satisfaction* was an essential part of the cluster of variables in the model. It showed that the individual singer evaluated his specific performance during the concert as part of the context of typical experiences during singing. It is interesting that both short-term responses about specific performance and long-term views about singing combined to predict IgA change. It is likely that singing experiences that were associated with higher levels of IgA involved feelings of satisfaction with performance. As an individual variable on the SRF, satisfaction correlated significantly with IgA ($r = .401$, $p < .027$).

Although the model that has been developed contains a number of intuitively satisfying components, the results should be interpreted with caution because of limitations associated with verbally scaled methods and physiological measurements whose diurnal rhythms are not yet understood.

Discussion

Three findings in this study supported the prediction that choral singing, particularly in performance, was associated with change in IgA level. The first finding was that IgA level increased in all three conditions and increased the most after performance, by a mean increase of 2.4 times over

baseline (confirming Hypothesis 1). A second finding was that a multiple regression model consisting of a specific set of psychological states generally associated over the long-term with performance singing strongly predicted IgA change after performance (confirming Hypothesis 3). A third finding was that singers' immediate, short-term evaluation of satisfaction with performance singing was part of the predictive model. Thus, our hypothesis that singing perceptions mediate the relationship of singing to physiological response was confirmed, at least with respect to public performance. Although this evidence does not speak to causality, as we cannot say which (if either) comes first, psychological state or physiological response, the findings suggested that mental and bodily responses were highly interrelated during singing. Although the findings for cortisol were significant in the expected direction for rehearsals (Hypothesis 2), they increased unexpectedly after performance. However, mean levels obtained for cortisol were too low to draw secure conclusions or relate these data to the measurements of the psychological instruments.

The results showed, in general, that performances were associated with both positive and negative emotions more than the rehearsals were. These data enabled us to refine our model of how emotions mediate between singing and the immune system indicators. The singers' ratings suggested that positive emotions during performance singing contributed to the increased levels of IgA that were found relative to baseline levels. But, it was also clear that negative emotions were part of this equation. Anxiety, in particular, was part of the singing experience. This anxiety was probably associated with appearing on stage in public, that is, it was performance anxiety. Thus, immune system functioning is likely to be stimulated both as a response to anxiety and as a function of the pleasure of the singing experience. As singers generally rated the performance as highly satisfying, we may speculate that for the majority of singers the positive emotions overcame the anxiety during the course of the performance. As a result the performance was not rated as stressful; a rating of stressful would have indicated that the performance failed to overcome the natural performance anxiety.

Thus some evidence suggests that for this group performance singing is both an anxious and highly stimulating experience that leads adaptively to levels of positive feelings and satisfaction. This complex of experiences was associated with positive changes in IgA level. In the long-term, these adaptive changes may become identified with the means for overcoming life stress. An analysis of qualitative responses to open-ended questions on the SEES revealed that many singers (approximately 25%) specifically viewed the benefits of singing as highly useful in coping with stress, both with respect to the performance and in life generally. For example, one singer said: "When I'm stressed all I have to do is start singing and the tension is

gone." Another singer said: [During singing] "I've cried out personal loss and celebrated joys." Another singer wrote: "It is for me very healing. I can come into a rehearsal or performance 'world weary' and leave refreshed and renewed." And, finally one singer wrote: "It gives me something to focus my energy on that takes me away from the worries of the day job." Thus, for many singers it is likely that the stress-reduction powers of singing are considerable.

Although the results of the present study suggest that significant interrelations exist between choral singing and immune response, future research should use a control group. We have envisioned various designs for future research, including comparing a group of orchestral wind instrument performers as a control group, perhaps in conjunction with a sample of choral singers performing the same piece of music. However, a more interesting experimental design might induce changes in singers' perceived levels of anxiety during singing and before, during, and after performances. For example, anxiety might be artificially stimulated by arranging for singers to be "late" in arriving for rehearsals, or by interrupting rehearsals, or by introducing criticism after the performance. These experimental conditions would be used to inform our model of singing as an adaptation to performance anxiety. Under these conditions, it would be predicted that increased perceived anxiety in singing would be associated with lower positive changes or negative changes in IgA when compared with groups who sang under normal conditions.[5]

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