

## Effects of Choir Singing or Listening on Secretory Immunoglobulin A, Cortisol, and Emotional State

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*The present study investigates the effects of choir music on secretory immunoglobulin A (S-IgA), cortisol, and emotional states in members of a mixed amateur choir. Subjects participated in two conditions during two rehearsals 1 week apart, namely singing versus listening to choral music. Saliva samples and subjective measures of affect were taken both before each session and 60 min later. Repeated measure analyses of variance were conducted for positive and negative affect scores, S-IgA, and cortisol. Results indicate several significant effects. In particular, singing leads to increases in positive affect and S-IgA, while negative affect is reduced. Listening to choral music leads to an increase in negative affect, and decreases in levels of cortisol. These results suggest that choir singing positively influences both emotional affect and immune competence. The observation that subjective and physiological responses differed between listening and singing conditions invites further investigation of task factors.*

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**KEY WORDS:** singing; S-IgA; cortisol; emotion.

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## INTRODUCTION

Since the times of Plato, artists and scientists alike have been intrigued by the power of music to elicit strong emotional responses in humans and animals. The Greek philosophers were among the first in the Western culture to speculate about specific effects of music on bodily chemistry, and subjective feelings (Levman, 2000). For example, they were convinced that music benefited health and specific recommendations were formulated for using music therapeutically against mental and physical illness (Bruhn, 2000).

Empirical research over the past decades has addressed the psychophysiological effects of music listening with some emphasis on the responses of the autonomous nervous system (ANS) (e.g., Goldstein, 1980; Kreutz *et al.*, 2002a,b; Panksepp, 1995). In general, this research suggests that listeners' subjective experience is at least in part mediated by physiological responses to music stimuli (Bartlett, 1996). However, few studies were able to relate peripheral ANS responses to emotional experiences of music (Krumhansl, 1997; Nyclicek *et al.*, 1997). To date, this research did not yet provide reliable evidence for associations between music and health related ANS variables.

It appears well established that the ANS profoundly affects immune functions (Ader *et al.*, 1991). Most of the studies reviewed below have used secretory IgA (S-IgA) as a marker of immune competence (Stone *et al.*, 1987b). S-IgA is a protein considered as the body's first line of defense against bacterial and viral infections of the upper respiratory pathway (Tomasi, 1972). In particular, S-IgA was found more strongly influenced via the sympathetic rather than the parasympathetic branch of the ANS (Ring *et al.*, 1999). There is general consensus that S-IgA is specifically responsive to an individual's emotional state (Rein and McCraty, 1995). Increases of S-IgA were often observed in the context of positive and/or relaxing experiences (e.g., Green and Green, 1987; Stone *et al.*, 1987a), whereas S-IgA often decreased in studies on the emotional impact of stressful events (Martin and Dobbin, 1988) and intense physical effort (Mackinnon and Hooper, 1994).

Research on the effects of music listening on immune system and emotional stress are receiving increasing attention in behavioral medicine (Pratt and Spintge, 1996). Several studies have specifically looked at the relationship between music listening, subjective mood, and immune competence. Music listening may significantly influence immune functions via the ANS (McCraty *et al.*, 1996). In particular, S-IgA increased in the context of music listening and relaxation (Tsao *et al.*, 1992; see also Van Rood *et al.*, 1993). Individual music preferences and context factors appear to be important in mediating these effects. Therefore, music may benefit patients in individual treatment formats (e.g., McKinney *et al.*, 1997a). McCraty *et al.* (1996)

concluded that music listening may enhance the beneficial effects of self-induced positive mood on immunity.

McKinney *et al.* (1997b) found that the combination of classical music and spontaneous imagery led to significant decreases in beta-endorphin, a hormone which is believed to be related to emotional stress (Panksepp *et al.*, 1979). Gerra *et al.* (1998) extended these findings using classical and techno music. They found correlations between personality traits such as novelty-seeking or harm avoidance and musically-induced endocrine responses, also including beta-endorphin.

In lieu of increasing evidence that music listening may influence immune competence and physiological stress in adult listeners (Pratt and Spintge, 1996), there is no comparable research on the conceivably enhancing effects of more active musical behaviors than listening. For example, the emotional effect of singing on the organism of the singer is yet poorly understood, although singing is probably the most common everyday musical activity observable in all cultures (Nettle, 2000). In a pioneering study, Beck *et al.* (1999) looked at the endocrine effects of singing in professional chorale singers. The authors were interested in the effects of three singing conditions (two different rehearsals and one performance) on changes of S-IgA and cortisol. Cortisol is a hormone associated with emotional stress (Kirschbaum and Hellhammer, 1994). Beck *et al.* (1999) found strong increases of S-IgA in each condition. For instance, during the performance S-IgA levels went up by more than 350% in nearly 25% of the singers. Levels of cortisol, by contrast, were found to decrease in the rehearsal conditions only, but increased significantly during the performance. These results suggested different effects of singing on S-IgA and cortisol. A further finding of the Beck *et al.* study was that according to a multiple regression analysis several subjective measures associated with positive attitudes toward singing predicted changes in S-IgA. To explain these findings it was speculated that breathing patterns induced by singing as well as positive mood change might contribute to the observed S-IgA increases.

More recent studies tend to corroborate subjective positive mood effects and health benefits of singing in groups (Clift and Hancox, 2001; Grape *et al.*, in press; Unwin *et al.*, 2002; Valentine and Evans, 2001). However, it is yet not clear whether and to what extent the observed effects could be attributed to mere passive exposure to musical sound, rather than active physical engagement in singing. As shown above, listening to music alone may induce a variety of significant endocrine effects, even irrespective of subjects' musical training (Bartlett, 1996; McCraty *et al.*, 1996).

On the basis of these previous findings, the purpose of the present study was to compare subjective and physiological responses produced by group singing with those elicited by listening to music and to establish any

differences as subjects' processed the same musical materials in these conditions. It was hypothesized that both singing and listening enhance specific immune functions as well as they lead to positive changes of affective states. Therefore, we expected significant increases in S-IgA and subjective positive emotional state as well as significant decreases of cortisol and negative emotional state after singing and after listening to choral music, but that these effects were more pronounced in the singing condition.

## METHODS

### Participants

Thirty-one members (23 female) of an amateur choir participated in this study. Participants' age ranged from 29 to 74 years ( $M = 56.9$  years,  $SD = 14.8$  years). As assessed by a questionnaire, none of the participants reported smoking more than 10 cigarettes per day or drinking more than 5 alcoholic drinks per week. On a questionnaire, subjects did not indicate acute health problems with respect to respiration or cardiovascular system. All subjects gave informed consent individually.

### Design and Procedure

After informed consent was obtained from all participants, the experimental conditions for this study were realized in two sessions at the same location in the rehearsal room of a church at the regular time of that choirs' rehearsal between 6 and 7 p.m. The sessions were conducted 1 week apart and lasted for 60 min each. Participants were instructed not to take in any meals, or alcoholic drinks, and refrain from smoking within 1 h before the start of the rehearsal.

Before the first session started, each participant filled in a demographic questionnaire. Moreover, before each of the two sessions, a psychometric scale for the measurement of emotional state (Positive and Negative Affect Schedule, PANAS; Krohne *et al.*, 1996; Watson *et al.*, 1988) was completed. The PANAS consists of 20 items, 10 representing positive affect (e.g., "I feel fine"), and 10 items representing negative affect (e.g., "I feel depressed"). Participants were asked to mark each of the items on a scale from 1 ("very little or not at all") to 5 ("extremely") according to their current feeling. The PANAS was filled in once again at the end of each session. Also, at the beginning and at the end of each session, saliva was collected using a standard procedure (see next section).

### *Singing Condition*

The singing condition was initiated by a 10-min warm-up phase, in which various breathing, stretching, and vocalization exercises were performed. For the rest of the session, sections and pieces from Mozart's *Requiem* were rehearsed, and instructions by the conductor were given to the choir. Participants stood during the warm-up, whereas they remained seated for the rest of the time. Times of interruptions by the conductor were measured and approximated 10 min of the rehearsal time.

### *Listening Condition*

During the second session 1 week later, the pieces from Mozart's *Requiem* were presented from CD, and articles on singing from an eighteenth century encyclopedia of the arts (Sulzer, 1967) were read aloud. Participants were seated during the entire session. When music was played, they were instructed to listen to the music attentively *as if* they were engaged in singing. Moreover, it was ensured that listening to speech and music under the listening condition had the same proportion as singing and listening to the conductor under the singing condition.

### **Saliva Collection and Assaying**

Saliva was collected with Sarstedt Salivettes<sup>®</sup>. This device consists of a plastic tube containing a cotton wool swab. Subjects were asked to insert the swab into their mouth and were instructed not to swallow saliva for a 5-min period. Afterwards this cotton wool swab was placed back into the tube. Saliva samples were centrifuged at  $4000 \times g$  for 10 min and then were kept at  $-30^{\circ}\text{C}$  until assayed.

Measured parameters in saliva samples were immunoglobulin A, albumin, and cortisol. Albumin levels served both as an exclusion criterion for blood contaminated saliva samples and for correcting the S-IgA measures for effects of saliva flow density. Because albumin leaks passively into saliva from systemic sources, its concentration reflects mucosal membrane permeability. The ratio of S-IgA to albumin thus provides an indication of the local secretory immune response controlling for any serum leakage of IgA (Cripps *et al.*, 1991; Drummond and Hewson-Bower, 1996).

After thawing, saliva was analyzed for concentrations of S-IgA and albumin by use of a fully automated nephometric analyses (BN100, Dade Behring, Marburg, FRG). The assay protocol has been adapted to the expected range for saliva concentrations of S-IgA between 0 and 120 mg/dL

and albumin (0–27 mg/dL), respectively using highly specific monoclonal antibodies for human S-IgA and albumin (Dade Behring). Previous measures revealed extremely high intra- and interassay precision which can be expected in general for protein analysis and which justifies single measurements of samples in clinical practice.

Saliva cortisol was determined using a commercial luminescence-immuno assay (IBL, Hamburg, FRG) especially designed for saliva samples and approved by the Food and Drug Administration (FDA). Pipetting of standards, samples, and reagents was performed by a fully automated system (Labotech, Freiburg, FRG).

Luminescence units were read by use of an automatic luminometer (Beckmann, FRG). All samples were measured in duplicates with sufficient intra-assay precision (coefficient of variance,  $CV < 6\%$ ). All samples were analyzed with assays obtained from the same charge to reduce interassay variation, which was lower than 10%.

Saliva analyses were conducted at the lab of Prof. Dr. J. Hennig at the Department of Psychology, Justus-Liebig-University Giessen, Germany (for more details see: Hennig *et al.*, 1999).

### Data Analysis

Positive and negative affect sum scores were calculated for the two conditions. To determine any significant changes of positive and negative affect, two repeated measures analyses of variance (ANOVA) were conducted for each of the two scores. Similarly, S-IgA/albumin and cortisol values were submitted to two separate repeated measures ANOVAs. In all analyses, condition (singing versus listening) and time (baseline and after treatment) served as independent variables. In addition, to determine statistical relationships between subjective and physiological changes, Pearson's product moment correlations were calculated.

## RESULTS

### Psychological Measures

Mean scores of positive and negative affect ratings before and after the two conditions are presented in Table I. Data from three subjects were not included due to large proportions of missing values.

An ANOVA for positive affect values indicated no significant main effect of condition,  $F(1, 27) = 3.08$ ,  $p = 0.09$ , or time,  $F(1, 27) = 1.30$ ,

**Table I.** Means (and Standard Deviations) of Positive and Negative Affect Ratings for the Two Experimental Conditions at Baseline and After Treatment

	Positive affect		Negative affect	
	Baseline	After treatment	Baseline	After treatment
Singing	2.86 (0.51)	3.15 (0.64)	1.31 (0.4)	1.18 (0.24)
Listening	2.85 (0.67)	2.79 (0.81)	1.23 (0.25)	2.20 (0.31)

Note. Scores of each scale were divided by the number of items.

$p = 0.26$ . However, there was a significant interaction between time and conditions,  $F(1, 27) = 6.41, p < 0.02$ . Follow-up Tukey’s HSD tests of simple effects revealed that positive affect increased significantly after singing ( $p < 0.05$ ), but not after listening. An ANOVA which addressed negative affects scores revealed highly significant main effects for condition,  $F(1, 27) = 95.71, p < 0.001$ , time,  $F(1, 27) = 113.57, p < 0.001$ , and a significant interaction between the two factors,  $F(1, 27) = 145.91, p < 0.001$ . Post hoc Tukey’s HSD-Tests of simple effects indicated a significant decrease of negative affect after singing ( $p < 0.05$ ), and a significant increase of negative affect after listening ( $p < 0.05$ ).

**Physiological Measures**

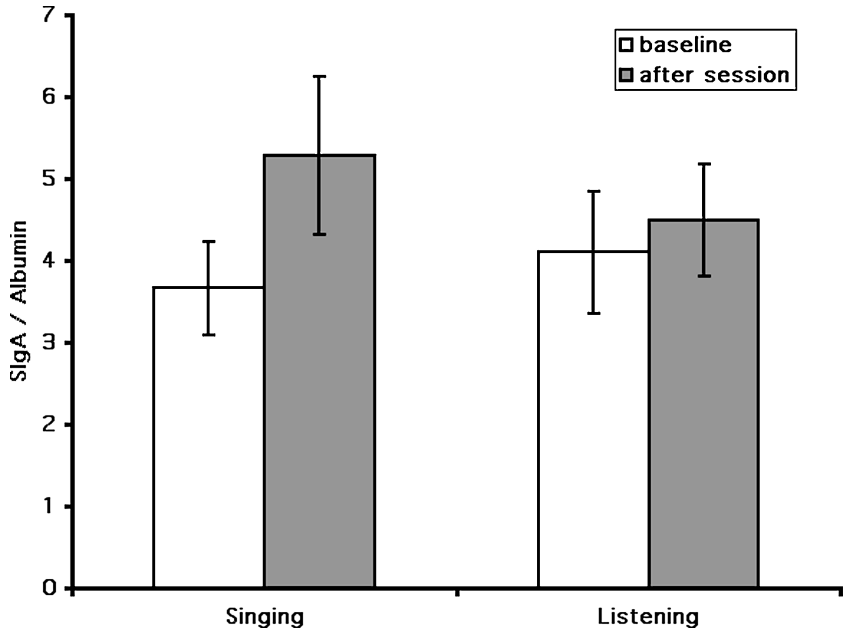
Table II presents mean S-IgA/albumin and cortisol values at baseline and after treatment.

An ANOVA of S-IgA/albumin values revealed a highly significant main effect of condition,  $F(1, 30) = 10.41, p < 0.005$ , but no significant main effect of time,  $F(1, 30) = 0.24, p = 0.62$ . As predicted, there was a significant interaction between time and condition,  $F(1, 30) = 4.32, p < 0.05$  on S-IgA/albumin. Follow-up Tukey’s HSD-Tests of simple effects indicated a highly significant increase of S-IgA/albumin for the singing condition ( $p <$

**Table II.** Means (and Standard Deviations) of S-IgA/Albumin and Cortisol Values Before and After Treatment for the Two Experimental Conditions

	S-IgA/albumin		Cortisol [ng/mL]	
	Baseline	After treatment	Baseline	After treatment
Singing	3.66 (3.15)	5.28 (5.26)	0.75 (0.67)	0.59 (0.48)
Listening	4.10 (4.20)	4.49 (3.78)	0.81 (0.61)	0.48 (0.27)

Note. S-IgA/albumin is without unit because the units for both parameters are identical (mg/dL).



**Fig. 1.** Means and standard errors of S-IgA/albumin values before and after singing choral music and listening to choral music respectively.

0.005), but no significant changes for the listening condition ( $p = 0.79$ ). Figure 1 illustrates the significant condition by time interaction on mean S-IgA/albumin values.

An ANOVA, which addressed cortisol values, revealed a significant main effect of time,  $F(1, 30) = 10.30$ ,  $p < 0.005$ , but no further effects. However, since we had specific hypotheses considering different effects of singing versus listening on cortisol responses, we did pairwise comparisons of baseline and after treatment values for both conditions separately. These analyses indicated that cortisol decreased significantly from baseline to after treatment in the listening condition,  $F(1, 28) = 12.14$ ;  $p < 0.001$ , but did not change significantly in the singing condition,  $F(1, 30) = 2.56$ ;  $p > 0.1$ .

Pearson correlations between changes in subjective and physiological measures were calculated separately for the two conditions. Out of eight coefficients, only one turned out to be significant: changes of positive mood during listening correlated significantly with changes of cortisol levels,  $r = 0.40$ ,  $p < 0.05$ .



## DISCUSSION

This study demonstrated psychophysiological effects of choral singing and listening to choral music. We found different patterns of changes for S-IgA, cortisol, and subjects' emotional state with respect to the two experimental conditions. Singing led to a decrease in negative mood and an increase in positive mood and S-IgA, but did not affect cortisol responses. Listening on the other hand led to an increase in negative mood, a decrease in cortisol, and no significant changes in positive mood and S-IgA.

These results support the hypothesis, that choir singing influences positive emotions as well as immune functions in humans. They confirm previous findings which showed that singing influences subjective emotional states positively (Unwin *et al.*, 2002) and enhances the immune defence (Beck *et al.*, 1999). These studies corroborate the notion that musically-induced changes of S-IgA are mediated by subjective mood (McCraty *et al.*, 1996; Rein and McCraty, 1995).

Contrary to expectations, we observed a decrease of cortisol only for the listening condition but not for the singing condition. On one hand, decreases of cortisol levels suggest psychological deactivation, relaxation, and stress reduction. On the other hand, it is known that cortisol levels decrease during the waking hours of human subjects (Kirschbaum and Hellhammer, 1994). This decrease is relatively steep in the morning but slows down in the afternoon. Given the fact that in the present study all measures were taken between 6 and 7 p.m. it is rather unlikely that the observed decrease of about 60% within 60 min in the listening condition is only due to the diurnal effect. The observation that listening also led to an increase in negative mood suggests that the listening condition was at least partly experienced as unexciting, boring, and deactivating by our participants. This interpretation is very reasonable given the fact that the main objective for these amateur choirs is the production but not the reception of music. The singing condition on the other hand may have prevented against deactivation and decrease in cortisol. Moreover, Beck *et al.* (1999) showed that the performance situation (rehearsal versus public concert) influenced the direction of changes of cortisol levels, which decreased during the rehearsals but increased during the public performance. The authors reasoned that the latter were emotionally more demanding than rehearsals.

The observed decrease of cortisol levels following the listening period, while participants reported increased negative mood in the same condition, is consistent with previous work (Davis and Thaut, 1989), which indicated that music listeners can present contradictory responses on psychological and physiological measures. One possible explanation for this dissociation

is that music expressing negative emotions, e.g., grief or sadness, is often experienced as relaxing and soothing (Västfjäll, 2002).

Positive emotions increased after singing, and negative emotions increased after listening. Why did listening to the music not result in the same subjective responses as singing? Many people report that they enjoy listening to music in a group setting such as attending a public concert. But again, it must be kept in mind, that the primary goal of a regular choir rehearsal is to practice singing. The experimental intervention required by the listening condition, therefore, was in conflict with the routine rehearsal procedure, and with the intensions and expectations of the individual choristers. In addition, choristers are more focused on vocal control, watching the music sheet and the conductor, and listening to their fellow singers during singing than during listening. Finally, previous studies have addressed the affective impact of lyrics (Stratton and Zalanowski, 1994), but it is unclear, whether and to what extent the contents of the lyrics are differentially perceived during listening as compared to singing. We assume, that the emotionally negative connotations of the requiem might have had a stronger impact on affective responses during listening than during singing.

Limitations of the present study should be noted. First, in this study, there was only one large piece of classical music included. Thus it remains to be seen, whether our findings can be generalized across different styles and genres of music as well as across the selection method of the musical materials (Thaut and Davis, 1993). Second, as this study was conducted with choral singers in a group setting, questions arise as to whether similar effects may be found in solo singing (Valentine and Evans, 2001). Third, we did not control for physical activity, which is known to influence mucosal immune system responses (Mackinnon and Hooper, 1994). For our subjects singing was physically more demanding than just listening. Future research should investigate whether and to what degree the observed effect of singing on S-IgA might be explained by different degrees of physical activity.

Finally, it seems worth to note that humans are not the only species to exhibit relationships between singing and immune functions (Duffy and Ball, 2002). One might speculate, that similar relationships as in this study may be detected in other primates, as vocal production in these species is directly related to emotional affect and stress-regulation (Grossmann, 2000; Hauser, 2000).

In sum, the present study shows that amateur group singing leads to increases in both positive affect and the production of salivary immunoglobulin A, a protein considered as the first line of defense against respiratory infections. It replicates previous work demonstrating an association between singing and immune function, and suggests a possible influence of musical behavior on well-being and health.

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