universität wien

DIPLOMARBEIT / DIPLOMA THESIS

Titel der Diplomarbeit / Title of the Diploma Thesis

„Hormonal effects of group singing: Oxytocin, Cortisol and Testosterone“

verfasst von / submitted by

Julia Gahr

angestrebter akademischer Grad / in partial fulfilment of the requirements for the degree of

Magistra der Naturwissenschaften (Mag.rer.nat.)

Wien, 2018 / Vienna, 2018

Studienkennzahl lt. Studienblatt / degree programme code as it appears on the student record sheet:	A 190 445 344
Studienrichtung lt. Studienblatt / degree programme as it appears on the student record sheet:	Lehramtsstudium UniStG UF Biologie und Umweltkunde UniStG UF Englisch UniStG
Betreut von / Supervisor:	Univ.-Prof. William Tecumseh Sherman Fitch, PhD

ACKNOWLEDGEMENTS

I am grateful to the Musisches Zentrum Wien (“Music Center Vienna”), which generously provided their practice rooms for the purpose of this study.
Furthermore, I am grateful to the choir “Neue Wiener Stimmen” and their director Christoph Wigelbeyer as well as the founder Jürgen Partaj for their support in making it possible to have the experiments during the actual rehearsal times. Moreover, I would like to thank Doris Geml for her kind support in organizing the where and when of the experiments. I would also like to acknowledge Daniel Bowling and Marisa Hoeschele for mentoring me.

Furthermore, I would like to personally thank the following people who played a significant role in supporting me with this investigation: Barbara Gahr, Walter Gahr, Clemens Gahr, Johanna Gahr, Henriette Wagner, the Vadi family, Marie Kalender, Stephan Herzog, Roman Schmid, Wolfgang Steiger, Julia Ritter and Manuel Punz.

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Abstract

Communal singing can be found in all known cultures and appears to have numerous positive effects on the human body. Not only has it shown to improve general well- being and mental health, it has also been related to facilitating group-cohesion. These effects may be based on endocrinological responses to communal singing. Oxytocin (associated with social bonding) and cortisol (stress) have previously been considered to play a key role in this matter. The aim of this study was to explore the roles of vocal mode (song vs. speech) and social context (group vs. solo) in triggering endocrinological responses to group singing. The experiment was conducted in the naturalistic setting of a choir rehearsal (N=71) and saliva samples were obtained before and after four conditions (group singing, solo singing, group speech and solo speech) for the measurement of oxytocin and cortisol, as well as testosterone (in men). In addition, subjects evaluated their affect (Positive and Negative Affect Schedule), closeness to their group (Inclusion of other in the self scale) and ten volunteers' heartrates were measured. Across all conditions cortisol and oxytocin concentrations were reduced significantly. Linear mixed model analysis indicated a significant effect of vocal mode on oxytocin and on testosterone levels, revealing that singing had a preservative influence on these hormones. In addition, oxytocin changes were generally more evident in men. Heart rates did not explain these hormonal changes, showing a general level of physiological arousal that was similar across all but the solo speech condition. Finally, communal singing induced the strongest improvements in mood and feelings of community. These results demonstrate the beneficial social and emotional effects of singing in humans and give an insight into the underlying hormonal mechanisms.

Keywords: group singing, communal singing, oxytocin, testosterone, cortisol, PANAS, IOS

1. Introduction

Music is a phenomenon that appears to have existed since the prehistoric times (Moore, 2015). Present in all known cultures, music plays a particularly important role in situations involving groups of people and heightened emotion (Unwin, Kenny & Davis, 2002). These include various public activities and rituals, often related to group identity (e.g. national anthems, community dancing), festivals (e.g. weddings, harvest), religion and spirituality (e.g. hymnal singing), conflict and war (e.g. military drills and marches) and physical labour (e.g. food production and preparation) (McNeill, 1995; Moore, 2015). Across these situations musical engagement appears to play a critical role in inspiring group cohesion and solidarity (McNeill, 1995), which has in turn been used to support an adaptive account of music in human evolution (Roederer, 1984). For example, Freeman (2000) writes that the biological significance of music (and dance) lies in its ability to strengthen feelings of unity and trust in large groups, helping to extend and strengthen social bonds beyond the nuclear family. The implication is that stronger bonds increase fitness by enhancing cooperation and efficient collective action, for example, in acquiring and defending resources (Huron, 2001). Individuals in groups that regularly engage in musical activity are thus hypothesized to have a biological advantage over those in groups that do not (Huron, 2001).

Perhaps the most basic form of social musical activity is group singing. Previous research has shown that group singing has a positive impact on general wellbeing, improving mood (Valentine & Evans, 2001; Kreutz, 2014; Unwin, Kenny & Davis, 2002; Pearce et al., 2016), strengthening mental health (Clift & Morrison, 2011; Kwan & Clift, 2017; Judd & Pooley, 2014; Dingle et al., 2012) and decreasing sensitivity to pain (Kenny & Faunce, 2004; Weinstein et al., 2016). Many studies have also demonstrated that group singing specifically enhances socially cohesive attitudes and behaviours (Welch et al., 2014; Faulkner & Davidson, 2006).

For example, Anshel and Kipper (1988) examined the role of music and activity in stimulating trust and cooperation between individuals, comparing the effects of active and passive musical conditions (group singing and group listening) with active and passive non- musical conditions (group reading and group film viewing). Their results showed significant increases in trust following the musical but not the non-musical conditions (Anshel & Kipper, 1988). More recently, Pearce, Launay and Dunbar (2015) investigated social bonding over time in previously unacquainted participants attending weekly singing or crafts classes for seven months. They found that singing functioned as an 'ice-breaker', eliciting feelings of closeness between participants in the singing class more quickly than in the crafts class (Pearce, Launay & Dunbar 2015). Similarly, Weinstein et al. (2016) found that even a single group singing session stimulates feelings of connection between strangers, and that these feelings are comparable to those observed between individuals in more established singing groups. Interestingly, group singing may stimulate social closeness regardless of whether individuals are in a cooperative or a competitive context. Pearce et al. (2016) found that group singing not only increased feelings of closeness between the members of a fraternity choir, but also towards their opponents in a singing competition. Consistent with an adaptive account of music based on social cohesion, there is thus considerable evidence that group singing facilitates social bonding. However, the physiological mechanisms by which this facilitation occurs remain obscure (Tarr, Launay & Dunbar, 2014).

One way to investigate these mechanisms is through an analysis of endocrinological changes in the body assayed through changes in the concentration of specific hormones in saliva. Saliva sampling, as opposed to blood sampling, provides a non- invasive and relatively stress-free sampling method, particularly suited to the assessment of behaviour in naturalistic contexts such as choir rehearsals (Gröschl, 2017). One hormone that has been considered in relation to social bonding is oxytocin, a neuropeptide produced primarily in the neurons of the hypothalamus that project to various locations in the brain and spinal chord as well as to the posterior pituitary gland, from which it is released into peripheral circulation (Norman & Henry, 2015). Oxytocin appears to play an important role in social bonding, facilitating affiliation and promoting social cognition (Heinrichs, van Dawans & Domes, 2009). For example, intranasal administration of oxytocin has been found to increase trust among humans playing an economic game (Kosfeld et al., 2005). Presently, studies examining oxytocin in the context of music remain inconclusive, with some showing decreases and some showing increases from before to after engaging in a musical task. Keeler et al. (2015), for example, examined oxytocin in a vocal quartet (N=4), comparing changes in salivary concentration from before to after standard and improvised singing performances. Their results showed a significant increase after improvised but not standard performance, which the authors argued reflects the critical role of "behaviours conducive to social bonding, such as listening, responding, spontaneous communication, eye contact, and cooperation" in improvisation (Keeler et al., 2015, p. 7). Similarly, Grape et al. (2003) found increased salivary oxytocin levels in students from before to after a private singing lesson, where they interacted with their singing instructor (Grape et al., 2003). Kreutz et al. (2014) found a significant increase in the salivary oxytocin of choir members from before to after a rehearsal, whereas a control condition where members chatted with friends about their lives did not lead to a significant oxytocin response. Contrastingly, Fancourt et al. (2016) found significant decreases in oxytocin concentrations following a 70-minute choir rehearsal. In a recent study similar to that performed here, Schladt et al. (2017) examined the effects of social context by comparing group singing and solo singing. Similar to Fancourt et al. (2016), they found significant reductions in salivary oxytocin from before to after a group rehearsal that were not apparent following solo rehearsal. This study, however, lacked a non-musical control condition.

A second hormone relevant to understanding physiological responses to group singing is the steroid hormone cortisol. Cortisol's actions are sustained through the Hypothalamic-pituitary-adrenal (HPA) axis and its release is stimulated by physical and/or emotional stress. (Norman &

Henry 2015). Overall, previous research on singing and cortisol associates increases with performing in front of an audience (Beck et al., 2000; Fancourt, Aufegger & Williamon, 2015), but decreases with singing in leisure/rehearsal situations (Beck et al., 2000; Fancourt, Aufegger & Williamon, 2015; Schladt, 2017). For example, two studies found significant reductions in cortisol following choir rehearsals (Beck et al., 2000; Schladt, 2017). Beck et al. (2000) collected saliva before and after two rehearsals of a professional choir and observed an average decrease of ~30% in cortisol concentration (Beck et al., 2000). Moreover, Schladt (2017) compared the effects of group and solo singing on salivary cortisol levels and found significant reductions after both conditions, but a greater decline after the group rehearsal. These results support the hypothesis that group singing in non-performance contexts reduces stress.

A third hormone, which has not previously been examined in the context of group singing, is testosterone. Testosterone is a steroid hormone synthesized in the testes, the adrenal glands and the ovaries (Eisenegger, Haushofer & Fehr, 2011). Besides being essential to the development of male primary and secondary sexual characteristics, testosterone appears to play an important role in behaviours associated with seeking and maintaining social status, such as aggression, stress and competition (Eisenegger, Haushofer & Fehr 2011). While testosterone has been found to increase in competitive situations, low testosterone has been linked to positive social interactions, for example between partners or friends (Anders, Goldey & Kuo, 2011). At present research on testosterone responses to music is limited. Two studies have found significant changes in salivary testosterone concentrations after listening to music for 30 minutes, with men experiencing testosterone reductions and women experiencing testosterone elevations (Fukui, 2001; Fukui & Yamashita, 2003). The authors suggest that these effects reflect the capacity of music to regulate sexual and aggressive behaviour (Fukui, 2001), which may in turn have important effects for the cohesion of large groups.

In sum, current literature indicates that singing, especially communal singing has the ability to accelerate social bonding and foster relaxation, which may be related to release of oxytocin and cortisol, respectively. However, available research does not address the question of whether it is the musical or the social component in a group setting that stimulates endocrinological responses. Accordingly, here we extend these studies by examining the effects of musicality and social context on salivary oxytocin, cortisol and testosterone in a group singing paradigm. Saliva samples were taken before and after four conditions: group singing, solo singing, group reading and solo reading. In addition, we measured changes in mood and feelings of social belonging with questionnaires, and examined heart rates in a subset of participants. If music is key to the endocrinological effects of group singing, then these effects should be greater in singing conditions than speaking conditions.

2. Materials and Methods

2.1. Participants

The participants in this study were 71 members (45 female; 26 male) of the Jeunesse youth choir in Vienna, Austria. They ranged in age from 17 to 28 years (mean = 23.11, SD = 2.6). This choir is organized around the Austrian academic semester, meeting once per week under the direction of a professional voice instructor to develop a repertoire of songs to be performed publically at the semester's end. This study was conducted half-way through the Winter semester, so that choir members had been singing together for at least two months. No participants were professional singers.

2.2. Experimental Design

We tested effects of two factors, social context (group vs. solo) and vocal mode (song vs. speech), resulting in four experimental conditions: group-song, group- speech, solo-song and solo-speech. All conditions followed the same 50-minute protocol, with a 20-minute core of activity preceded and followed by a 5-minute survey and 10 minutes of saliva sample collection (Figure 1).

Figure 1. Experimental protocol. Diagram depicting the order and duration of activities in an experimental session.

All experiments took place on Monday evenings shortly before or during regularly scheduled choir practice (18:00-20:45) at the Musisches Zentrum Wien ("Music Center Vienna"). The group-song and group-speech conditions took place on the same day, with the 68 participants in attendance being split into two groups balanced for sex and vocal range (song: N=37, 15 male; speech: N=31, 10 male). In the group- song condition, participants stood and sang in chorus for 20 minutes led by the choir director, starting with 5-minutes of vocal warm-up and followed by 15 minutes of singing songs from the choir's repertoire. The director was asked not to interrupt the singers during singing and to keep the pauses between songs as short as possible (~30 seconds). In the group-speech condition (led by JG), participants stood and spoke the lyrics to the same songs. Prior to starting, JG instructed participants to speak the lyrics like a story, without following a particular tune or rhythm. Speaking the song lyrics did not take as long as singing them, so JG led the speakers through the repertoire ~1.5 times until 20 minutes had elapsed. The solo conditions took place over six Mondays, half before and half after the group session, to control for potential order effects. The solo conditions matched the group conditions as closely as possible, with the exception that participants performed by themselves in an acoustically isolated practice rooms. A total of 44 choir members participated in solo sessions (all but 3 also participated in the group session): 25 were assigned to sing (11 male) and 19 to read (7 male). Choir members who participated in both the group and solo sessions were always assigned to the same vocal mode. The protocol for the solo sessions was the same as that used in the group session. The instructions for the solo conditions were provided in written form.

2.3. Saliva Collection

Saliva samples were collected using the passive drool technique. Participants leaned forward and allowed saliva to flow passively through a tube (Saliva Collection Aid; Stratech Scientific LTD) into a cryovial (3.5 ml; Stratech Scientific LTD). The drooling periods for sample collection were 10 minutes in duration. To reduce the risk of contamination, participants thoroughly rinsed their mouths with water 5 minutes prior to the start of each drooling period. Additionally, participants were asked not to consume any major meals for at least one hour prior to experiments and to avoid foods or drinks containing acid, sugar or caffeine during the 15 minutes before the experiment. After sample collection, the cryovials were immediately sealed and put on dry ice in a small cooler. The frozen samples were transferred (within 1-2 hours) to a freezer for storage at -80°C until the time of assay.

2.4. Hormone Assays

Samples were thawed and centrifuged at 1660g for 20 min. The supernatant was aliquoted in microcentrifuge tubes and stored frozen until analysis except for oxytocin, which was processed immediately. To quantify cortisol, oxytocin (OT) and testosterone (T; only in men) commercial Elisa kits were used (cortisol, Demeditec, Cat Nr. DES6611; oxytocin, Arbor Assay, Cat Nr. K048; testosterone, IBL, Cat Nr. RE2631). All standards and samples were run in duplicates in all assays.

Cortisol. Aliquotes were thawed, vortexed, and centrifuged for 20 min at 1660 g. The concentration of cortisol in the supernatant was determined following the manufacturer's instruction. The interassay coefficient of variation (CV) was lower than 8% and the mean intraassay CV was 4.49% (SD=5.05). Detection limit of the assay was 0.024 ng/ml.

Oxytocin. To quantify OT, an extraction procedure was first performed following the manufacturer's instructions. 300ul saliva were diluted 1:1.5 with Extraction solution (provided by company), then vortexed and incubated on shaker for 90 min. After centrifugation for 20 min at 4 C at 1660 g the supernatant was transferred into a glass tube and dried down under N_2 stream at 37 C. The extracts were re-suspended in 250ul Assay buffer to proceed with quantification of OT. The assays were conducted following the protocol provided with the kit. The interassay CV was <8% and the mean intraassay CV was = 4.64% (SD = 6.1). The detection limit was set 40 pg/ml.

Testosterone. Sample were thawed, vortexed, and then centrifuged at 3000 g for 10min. Supernatant was used for further analysis of T following the Assay protocol of the company. The interassay CV was <11% and the mean intraassay CV was = 7.76% (SD = 9.69). Detection limit was 10.0 pg/ml.

2.5. Surveys

Self-perceived affect was assessed by administering a German version of the Positive and Negative Affect Survey (PANAS) (Breyer & Bluemke, 2016) before and after each experimental manipulation (see Figure 1). The PANAS consists of two 10- item mood scales focusing on positive and negative affect (Watson & Clark, 1988). Positive affect was described with adjectives such as enthusiastic, active and inspired; Negative affect was described by adjectives such as distressed, hostile and ashamed (Watson & Clark, 1988). Respondents indicated to what extent each adjective reflected their current mood using a 5-point scale from 1 (“not at all”) to 5 (“completely”). The PANAS data was used to compute a measure of “total affect”, equal to the sum of ratings for items on the positive scale minus the sum of ratings for items on the negative scale. For the group conditions, participants were also asked to rate how close they felt to their colleagues, using the Inclusion of Other in Self (IOS) scale (Aron, Aron & Smollan, 1992). The IOS scale asks respondents to choose from several diagrams depicting two circles overlapping to various degrees in representation of different degrees of emotional closeness between individuals.

2.6. Arousal

Heart rate was used as a measure of physiological arousal during the experimental manipulations. For the group session, heart rate was simultaneously recorded from 10 volunteers using the Polar Team Pro wireless sensor system (Polar, Kempele, FI). In the configuration used here, this system consisted of 10 Polar H7 heart rate sensors each held in place on a participant’s chest by an elastic strap. These sensors wirelessly transmit pulse data (sampled at 10 Hz) to a receiver for storage. The individuals selected to wear the sensors included 5 singers (2 male) and 5 speakers (3 male), roughly matched in height and weight. Apart from one female speaker, all individuals assessed in the group session were also assessed in a solo session. The missing female speaker was ill on the day of her scheduled solo session and was replaced by another female speaker of similar height and weight.

2.7. Statistics

The change in each hormone (or survey) from sample #1 to sample #2 was expressed as a delta score (Δ = [sample #2-sample #1] / sample #1 x 100). Experimental effects were assessed using linear mixed effects (LME) models to predict delta scores as a function of fixed effects for social context (group vs. solo), vocal mode (song vs. speech) and sex (male vs. female), and a random effect for subject ID (1-71). The significance of each fixed effect was tested using a likelihood ratio test to compare version of the models including and excluding it (Winter 2013; Levy 2014). Post-hoc tests for comparisons of interest were performed using t-tests (with Bonferroni corrected p-values). Additional statistical tests were performed using Mann-Whitney U-tests or Wilcoxon signed-rank tests as appropriate. All statistics were calculated in R Studio (v1.0.153; R Core Team, 2015). The 'lmer' function from package 'lme4' (v1.1-13; Bates et al., 2015) was used to construct models, the 'anova' function from package 'car' was used to conduct likelihood ratio tests (v2.1-5 Fox et al., 2013), the 'contrast' function from package 'lsmeans' to conduct post-hoc tests (v 2.27-61; Lenth, 2016), and 'wilcox.test' from package 'stats' was used to perform Mann-Whitney and Wilcoxon tests (v3.3.3; R Core Team, 2015).

3. Results

3.1. Salivary Hormones

A total of 224 saliva samples (in 112 sample #1 – sample #2 pairs) were collected across the four experimental conditions. Each sample consisted of 1-3 ml of saliva. A small number of samples could not be assayed for all three hormones due to insufficient test volume and/or corruption with extraneous material (e.g. mucus, lipstick). Because the analyses focused on paired samples, the exclusion of one sample necessitated the exclusion of its pair. In total, 7 pairs were excluded from the cortisol analysis (6.25% of all samples), 3 pairs were excluded from the oxytocin analysis (3.57%), and 4 pairs were excluded from the testosterone analysis (9.3% of all male samples).

Cortisol. A total of 210 saliva samples (105 pairs) were assays for cortisol. For the analysis of delta scores, one additional pair of samples (from a male solo singer) was excluded because of a 411% increase in cortisol, a full 24 standard deviations above the mean change observed across all other pairs. The following analyses thus included 104 paired saliva samples (from 69 unique individuals, 35 of whom participated in group and solo conditions). The results of the salivary cortisol analysis are shown in Figure 2. A clear pattern of decreasing cortisol was evident across conditions (sample #1 mean = 2.68 ng/mL, sample #2 mean = 1.89 ng/mL; mean Δ score = -27.8% [SD=17.8%]; $W = 5163$, $p < 0.0001$), with 94% of paired measurements showing a decrease. Beyond this global decrease, however, the LME analysis of cortisol Δ scores did not indicate any significant differences for social context (mean group Δ = -28.5%, mean solo Δ = -26.6%; $\chi(1) = 0.323$, $p = 0.57$), vocal mode (mean song Δ = -27%, mean speech Δ = -28.7%; $\chi(1) = 0.262$, $p = 0.609$) or sex (mean male Δ = -29%, mean female Δ = 27.1%; $\chi(1) = 0.333$, $p = 0.564$).

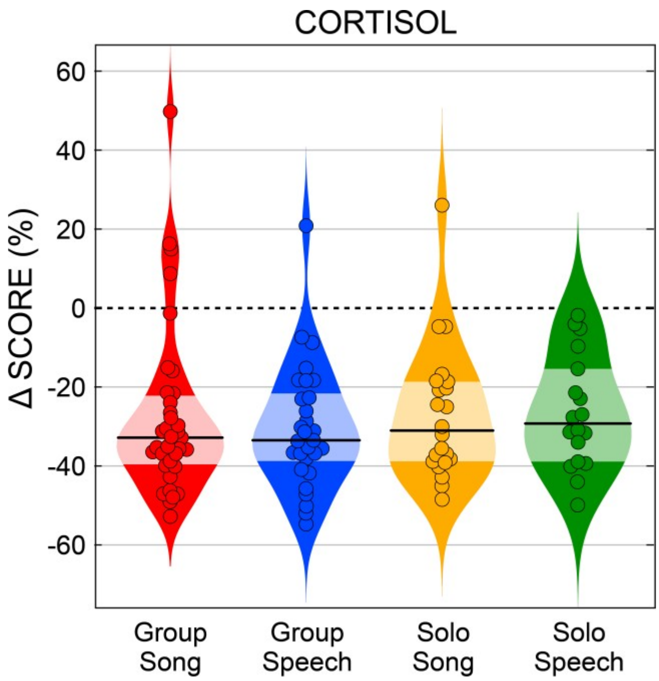

Figure 2. Salivary cortisol. Changes in salivary cortisol concentration from before to after the experimental manipulation in each of the four conditions. Each circle represents the delta score for one pair of measurements (calculated as [sample #2 – sample #1] / sample #1 x 100). For each condition, the data is overlaid on a violin plot of their estimated probability density (Matlab function 'violin.m'). Horizonal black lines represent medians; lighter-colored regions near the center of each violin represent interquartile range. Ns from left to right: 35, 29, 22 and 18.

Oxytocin. A total of 218 saliva samples (in 109 pairs) were assayed for oxytocin. No sample pairs were removed as outliers, however, a large number of samples (n=51) exhibited concentrations < 40 pg/mL, the global detection limit recommended by the assay manufacturer. Sample pairs for which both members exhibited concentrations < 40 pg/mL (n=16) were excluded from this analysis because it was not possible to detect any change in oxytocin. Each of the remaining sub-40 pg/mL samples only affected a single member in a pair and were thus retained. The following analyses thus included 93 paired samples (from 63 unique individuals, 30 of whom participated in group and solo conditions). The results of the salivary oxytocin analysis are shown in Figure 3. As with cortisol, a significant decrease in oxytocin was observed across conditions (sample #1 mean = 153.2 pg/mL, sample #2 mean = 85.8 pg/mL; mean Δ score of -14.3% [SD=39.5%]; Wilcoxon's W = 3392, p < 0.0001), with 70% of paired measurements showing a decrease. The LME analysis of oxytocin Δ scores indicated significant effects of vocal mode (song mean Δ = -4%, speech mean Δ = -24%; $\chi(1) = 8.31$, $p = 0.0039$), and sex (female mean Δ = -7.2%, male mean Δ = -24.7%; $\chi(1) = 6.73$, $p = 0.0095$), but not social context (group mean Δ = -16.5%; solo mean Δ = -10.9%; $\chi(1) = 0.547$, $p = 0.46$). Post-hoc tests comparing group-song and group-speech were significant, as were those comparing solo-song and solo-speech (ts[53.91] = -2.98, ps = 0.008; Bonferroni corrected for 2 tests).

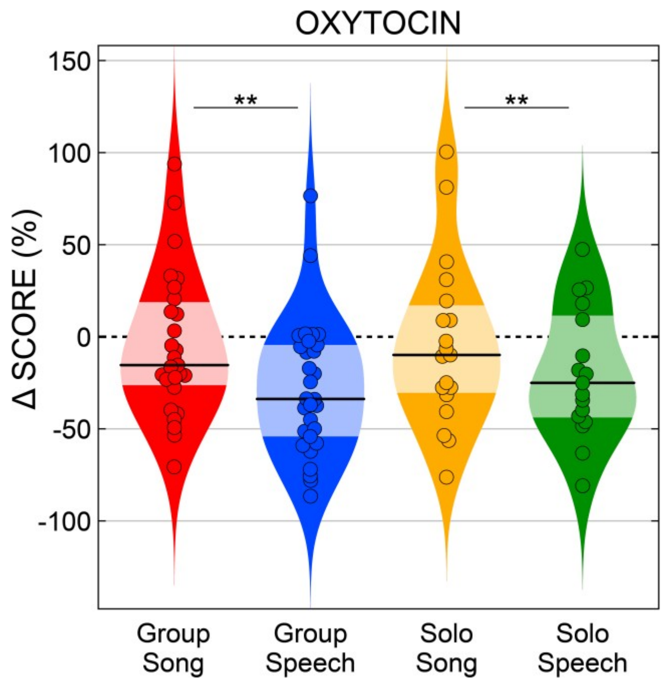

Figure 3. Salivary oxytocin. Changes in salivary oxytocin concentration from before to after the experimental manipulation in each of the four conditions. Format is the same as Figure 2. Ns from left to right: 27, 30, 19 and 17. ** = $p < 0.01$.

Testosterone. A total of 78 saliva samples (in 39 pairs; all from males) were assayed for testosterone. No sample pairs were removed as outliers. The following analyses thus included data from 39 paired samples (from 23 unique individuals, 16 of whom participated in group and solo conditions). The results of the salivary testosterone analysis are shown in Figure 4. In contrast to cortisol and oxytocin, no significant difference in testosterone was observed across conditions (sample #1 mean = 77 pg/mL, sample #2 mean = 72.7 pg/mL; mean Δ score = -5.4% [SD=23.1%]; Wilcoxon's W = 491, $p = 0.159$), with 59% of paired samples showing a decrease. The LME analysis of testosterone Δ scores indicated a significant effect of vocal mode (song mean Δ = +2%, speech mean Δ = -16.1%; $\chi(1) = 5.86$, $p = 0.0155$), but no effect of social context (solo mean Δ = -1.6%, group mean Δ = -8.4%; $\chi(1) = 1.217$, $p = 0.27$). Post-hoc tests comparing group-song and group-speech were significant, as were those comparing solo-song and solo-speech ($ts[20.4] = 2.56$, $ps = 0.037$; Bonferroni corrected for two tests).

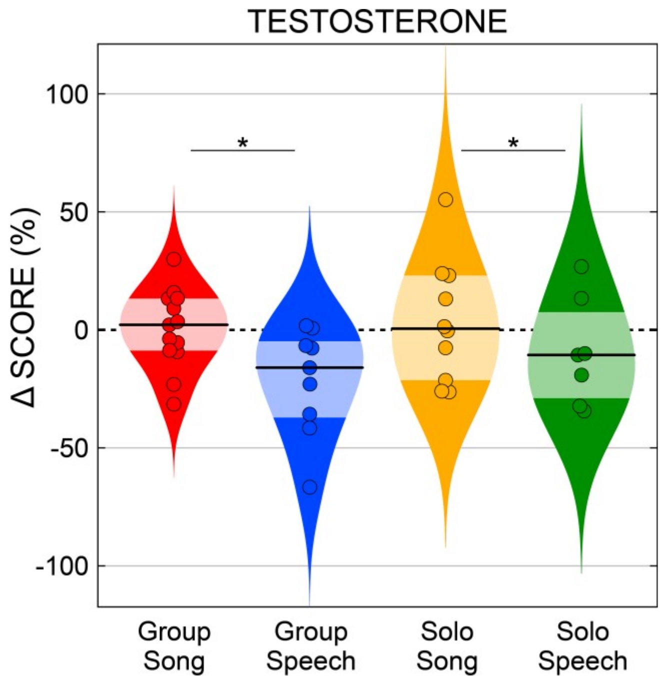

Figure 4. Salivary testosterone. Changes in salivary testosterone concentration from before to after the experimental manipulation in each of the four conditions. Format is the same as Figure 2. Ns from left to right: 13, 9, 10 and 7. * = $p < 0.05$

3.2. Surveys

Positive and negative affect schedule (PANAS). In parallel with the saliva samples, the PANAS was administered 224 times (in 112 sample #1 – sample #2 pairs) across the four experimental conditions. Three pairs were excluded due to incomplete responses, leaving 109 survey pairs for the following analysis. The results of the PANAS survey analysis are shown in Figure 5A. Total affect was generally positive and showed a small but significant increase across conditions (sample #1 mean = 15.1, survey #2 mean = 17.5; mean Δ score = +2.4 [SD=10.1]; $W=1736$, p =

0.0008), with 62% of paired surveys showing increases. The LME analysis of total affect Δ scores indicated a significant interaction between social context and vocal mode ($\chi(1) = 14.7$, $p = 0.0001$), with a significant difference between group-song and group- speech (mean Δs = +8.6 and -5.7 respectively; $t[96.22] = -6.88$, $p < 0.0002$) that was over 5x larger than that observed between solo-song and solo-speech (mean Δs = +3.7 and +1.2; $t[109] = -1.23$, $p = 0.44$; Bonferroni corrected for two tests).

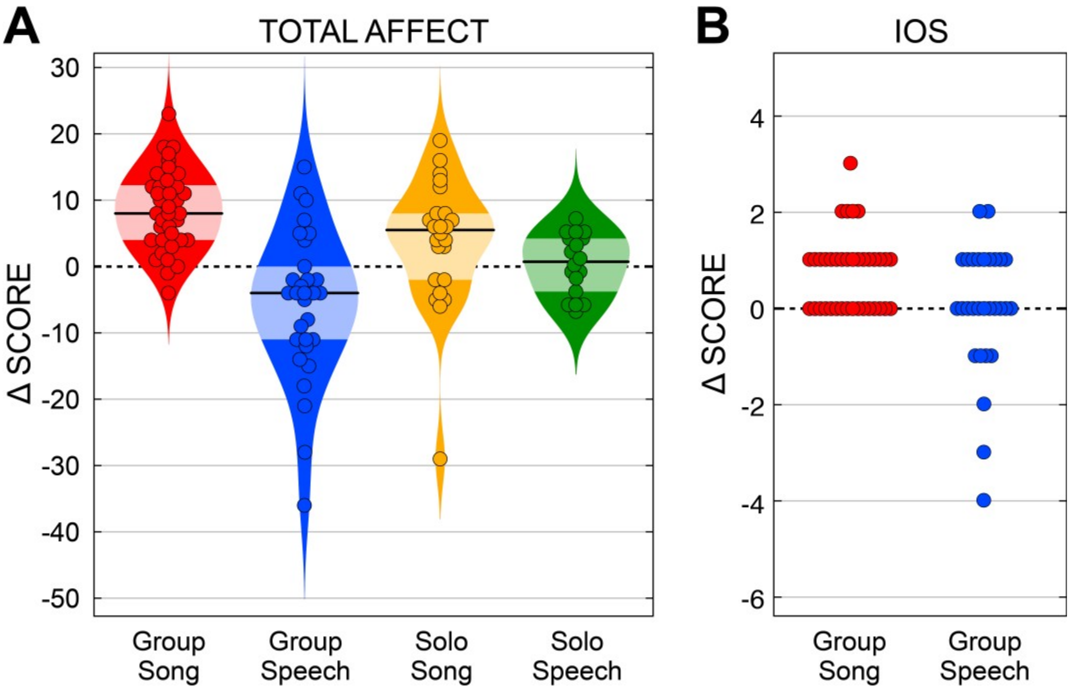

Figure 5. Surveys. (A) Changes in PANAS total affect from before to after the experimental manipulation in each of the four conditions. Ns from left to right: 37, 30, 24 and 18. (B) Changes in IOS scores before to after the experimental manipulation in the two group conditions. Ns from left to right: 37, 29. Format is the same as Figure 2. Violin plots omitted in B because they are not designed for discrete data.

Inclusion of the other in the self (IOS). The IOS survey was administered 68 times (in 34 pairs). Two pairs were excluded due to incomplete responses, leaving 66 survey pairs for the following analysis. Comparison of IOS Δ scores in group-song and group-speech showed a significant effect of vocal mode ($W = 1404$, $p = 0.0236$), with larger increases in the song condition than the speech condition (mean Δs = +0.73 and 0 respectively). At an individual level, this was reflected by the fact that Δs in the song condition either showed increases (57%) or did not change (43%), whereas Δs in the speech condition showed increases (38%), did not change (38%), or showed decreases (24%).

3.3. Arousal

The results of the heart rate analysis are shown in Figure 6. Heart rates were largely overlapping for the group-song, group-speech, and solo-song conditions (means = 99.4 BPM, 94.4 BPM and 92.8 BPM respectively), but lower in the solo reading condition (mean = 76.2 BPM). The LME model showed a significant interaction between social context and vocal mode ($\chi(2) = 6.93$, $p = 0.0312$), reflecting the similarity between group-song and group-speech ($t[13.78] = -0.64$, $p = 1$) and the difference between solo-song and solo-speech ($t[109] = -1.23$, $p = 0.0736$; Bonferroni corrected for two tests).

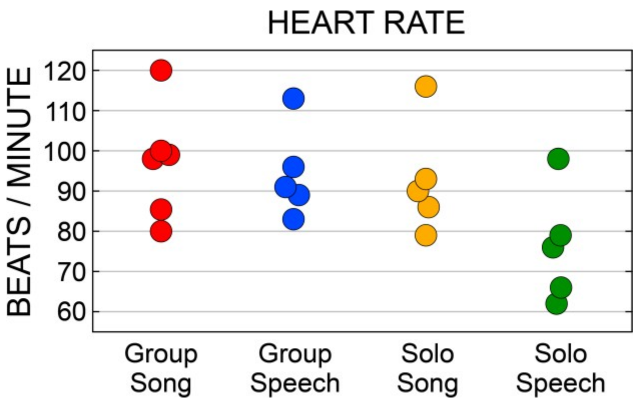

Figure 5. Heart rate. Mean heart rate in beats per minute calculated over the core 10 minutes of each experimental condition. Each circle represents the mean heart rate for one individual in one condition.

4. Discussion

This study demonstrated that 20 minutes of group singing can elicit a notable emotional and hormonal response. Not only did singing improve the participants' mood but it also strengthened feelings of social inclusion. We observed reductions in average cortisol and oxytocin levels across different conditions. Oxytocin reductions were greater after speech and generally more pronounced in the male cohort. Average testosterone levels were similar before and after singing but, like oxytocin, decreased more after speech. The heart rate analysis suggested a general level of physiological arousal that was similar across the group singing, group speaking and solo singing conditions, but lower in the solo speaking condition.

The surveyed emotional effects of group singing are in agreement with earlier studies using the PANAS questionnaire to evaluate mood (Kreutz et al., 2004; Weinstein et al., 2016; Pearce, Launay & Dunbar, 2015). Our results confirm the findings of Schladt et al. (2017) that group singing leads to a stronger increase in positive affect than solo singing. In contrast, group speech had a negative impact on mood, indicating an interaction between vocal mode and social context. Similarly, Kreutz (2014) found that group singing reduced negative feelings, but that chatting with friends (their control condition) did not. Furthermore, group singing significantly increased a feeling of community within the choir, which is also consistent with previous findings (Pearce et al., 2016; Weinstein et al., 2016; Pearce, Launay & Dunbar, 2015) and supports the hypothesis that musical activity facilitates social cohesion.

With respect to cortisol, our findings are in agreement with earlier studies showing significant decreases after group singing (Schladt, 2017; Beck et al., 2000; Fancourt, Aufegger & Williamon, 2015; Fancourt et al., 2016). However, while Schladt et al. (2017) noted a greater salivary cortisol decline after group singing than after solo singing, we did not find any significant effects of social context. Compared to our data, the cortisol decrease after group singing reported by Schladt et al. (2017) was larger, whereas the cortisol decrease after solo singing was smaller. This suggests that Schladt et al.'s (2017) group singing sessions were more relaxing and their solo sessions were less relaxing compared to ours. It is unclear which factors could have led to such differences, given the similarity of the experimental designs. One possibility is that our solo singing sessions took place in large, open practice rooms typically used for group sessions, whereas Schladt et al.'s (2017) solo sessions took place in small practice rooms. In support of this possibility, several of our participants commented positively on having the entire room to themselves. In terms of group singing, Schladt et al. (2017) may have managed to create a more natural choir rehearsal atmosphere than we did. A further difference in cortisol responses between our study and Schladt et al.'s (2017) is that they observed a sex-difference (larger decreases in men) whereas we did not. This is particularly surprising given the larger sample size and higher number of males in our study compared to Schladt' et al.'s (N=71, 26 male vs. N=38, 17 male respectively) and further highlights the highly contextualized nature of hormonal responses. A final point is that since cortisol is known to operate in a diurnal rhythm, the reductions we observed could potentially be an artefact of generally declining levels throughout the day (Weitzman et al., 1971; Miller et al., 2016), particularly given the lack of experimental effects. However, this is unlikely because of the large magnitude of the effects we observed (a decrease of 28% on average over 30 minutes). Assuming an average wake-up time of ~8:00, the average diurnal decrease in cortisol, expected over 30 minutes in the time window during which the experiment was conducted (17:00-19:00), would be approximately 8% (Miller et al., 2016).

There is an ongoing controversy in previous research about the effects of group singing on oxytocin. While two studies observed elevated oxytocin levels following group singing (Kreutz 2014; Keeler et al., 2015) two other studies found oxytocin reductions (Schladt et al., 2017; Fancourt et al., 2016), the latter being in line with our findings. The differences in these studies may be due to smaller sample sizes (e.g. N=4 in Keeler et al., 2016), different settings and problematic control conditions. Compared to our choir, where the majority of singers had worked together before and were sharing a common goal (concert at the end of the semester), Kreutz (2014) formed a choir for the purpose of his experiment only and also preferentially selected participants with little or no singing experience. These contextual factors are likely to have produced different group dynamics, which in turn may have altered hormonal responses. Furthermore, inexperienced individuals singing with strangers are likely to react differently than experienced individuals singing with friends. Pierrehumbert et al. (2010) hypothesized that in situations of social stress oxytocin is released alongside cortisol to promote pro-social behaviour and help down-regulate cortisol levels. Whereas this hypothesis may help explain the results of Kreutz (2014), it does not apply here, as we observed global reductions in both oxytocin and cortisol, as well as a dissociation in their responses. Vocal mode had a significant effect on oxytocin (greater decreases after speaking than singing) but not cortisol. Our results thus show that whereas all of our experimental manipulations decreased the cortisol mediated stress-response, only singing had a preservative effect on oxytocin levels. This data is consistent with the idea that the musical aspect of singing, whether performed alone or in a group, up-regulates oxytocin activity. Finally, we observed that oxytocin decreases were greater in males than females across the board, suggesting a sex difference in the sensitivity of the oxytocin system to the tasks performed here. One possible cause of this sex difference may be the use of oral contraceptives, as oestrogens appear to regulate baseline oxytocin levels (Taylor et al., 2006; Neumann & Landgraf, 2012). However, previous research suggests that contraceptives do not disrupt oxytocin responses to experimental manipulations (Jong et al., 2015; Pierrehumbert et al., 2010).

In terms of testosterone, we observed a significant effect of vocal mode, with the speaking conditions resulting in greater decreases than observed in the singing conditions. This pattern of results is parallel to that observed for oxytocin, suggesting that singing may also have a preservative effect on testosterone levels. In comparison, two studies found that passive listening to music led to declined testosterone levels, indicating that testosterone responses differ during active and passive musical activity (Fukui, 2001; Fukui & Toyoshima, 2013). Interestingly, we did not observe an effect of social context on testosterone, although it had been presumed that testosterone levels vary according to the social environment (Kornienko et al., 2016). Furthermore, there is evidence that testosterone interacts with cortisol to regulate social aggression (Montoya, Terburg, Bos & van Honk, 2011), reduce fear (Eisenegger, Haushofer & Fehr, 2011) and build relationships (Kornienko et al., 2016). The dual hormone hypothesis suggests that testosterone only enhances status-seeking behaviours when cortisol levels are low (Mehta & Prasad, 2015; Mehta & Josephs, 2010). In addition, higher testosterone and lower cortisol have been associated with increased individual popularity and connectedness to a team in sports (Ponzi et al., 2016; Edwards, Wetzel & Wyner, 2006). Thus, the preservative effects of singing on testosterone in men observed here, paired with the overall reduction in cortisol, may reflect a connection between singing and competitive/status-seeking behaviour. Considering the fact that the choir group was mostly female (2/3), such competitive behaviour could be related to courtship.

Finally, the analysis of the heart rate data suggests that the general level of physiological arousal is unlikely to account for the mood or hormonal changes that we observed. Heart rates were highly similar between the group singing, group speaking and solo singing condition, but lower in the solo reading condition. Thus, whereas differences in arousal tracked changes in oxytocin and testosterone in the solo conditions, they did not in the group conditions. The similarity of heart rates between the group singing and speaking conditions suggests that interacting with others may be sufficient to increase physiological arousal, regardless of vocal mode.

Before concluding, there are a few limitations to this study that need to be addressed. Although quantifying oxytocin in peripheral fluids has become popular in recent years, the extent to which peripheral oxytocin concentrations reflect oxytocin concentrations in the central nervous system is unclear (Valstad et al., 2017). While peripheral oxytocin is typically associated with physical reproductive functions, for example, in parturition and lactation, central oxytocin is usually associated with social behaviour (Gimpl & Fahrenholz, 2001). It has been shown that central oxytocin concentrations correlate positively with peripheral concentrations in response to many physiological stimuli, but it has also been suggested that the central/peripheral systems may behave independently in the absence of strong external stimuli (Neumann & Landgraf, 2012). In their review on this matter Valstad et al. (2017) conclude that a positive association between peripheral and central oxytocin concentrations is dependent on the experimental situation, being more likely to occur after experimental stress induction. Since our experimental manipulations induced hormonal responses, a peripheral-central correlation is plausible in the present context. Nevertheless, due to a lack of research and the fact that we did not use a classical stressor, we cannot make any strong inferences about central oxytocin levels. Turning to the experimental design, only a subset of the subjects could participate in both a group and a solo condition. Moreover, the subjects either participated in the singing or the speaking condition, meaning that we could not trace individual differences in hormonal response to different activities. Finally, future research should also consider testosterone levels in women as there might be sex-related differences in hormonal interplay and its influences on emotion and behaviour patterns (Montoya, Terburg, Bos & van Honk, 2011; Casto & Edwards, 2016).

In conclusion, this study has shown that singing can elicit stronger improvements in mood and social connectedness than non-musical activity, corroborating early work on the benefits of active musical participation. Our hormonal findings do not support the hypothesis that singing increases oxytocin activity to enhance social bonding in group contexts, instead showing that compared to non-musical activity, musical activity tends to preserve oxytocin activity regardless of whether performed alone or in a group. We observed a similar pattern in testosterone, with greater average decreases after the non-musical conditions. Finally, the cortisol results showed that group musical activity reduces stress, but not in a way that differs from the other activities examined here. Taken together, these results show that group singing improves mood and promotes social cohesion, thereby increasing the likelihood of harmonious group interaction, which could be beneficial when searching for food or mating partners, looking after offspring or defending the group against predators. At an endocrinological level, this appears to depend on the preservative effects of singing on oxytocin and testosterone (in males) paired with more general effects on cortisol.

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6. Appendix

6.1. Zusammenfassung

Gruppengesang ist ein Phänomen, das in allen bekannten Kulturen auftritt und zahlreiche positive Auswirkungen auf den menschlichen Körper zu haben scheint. So trägt Gruppensingen zu einer Verbesserung des allgemeinen Wohlbefindens und der psychischen Gesundheit bei. Außerdem scheint gemeinsames Singen den Zusammenhalt in einer Gruppe zu verstärken. Es wird vermutet, dass diesen Effekten endokrinologische Reaktionen zu Grunde liegen. Oxytocin, ein Hormon das mit sozialen Bindungen assoziiert wird, und Cortisol, das so genannte Stresshormon, könnten hierbei eine wichtige Rolle einnehmen. Die Studie sollte nun zeigen, ob der vokale (singen vs. lesen) oder der soziale (Gruppe vs. Solo) Aspekt der Auslöser solcher hormonellen Reaktionen ist. Das Experiment wurde im natürlichen Umfeld einer Chorprobe (N=71) durchgeführt. Vor und nach Gruppengesang, Sologesang, Gruppenlesen und Sololesen, wurden Speichelproben entnommen, die mittels ELISA (Enzyme Linked Immunosorbent Assay) auf Oxytocin, Cortisol und Testosteron (nur bei männlichen Probanden) getestet wurden. Des Weiteren, bewerteten die TeilnehmerInnen ihre Stimmung (Skala der momentanen positiven und negativen Affektivität, PANAS) und ihre Identifikation mit der Gruppe (Inclusion of the other in the Self – IOS). Bei zehn TeilnehmerInnen wurde zusätzlich der Puls gemessen. Die Ergebnisse zeigten eine generelle Abnahme der Cortisol- und Oxytocin-Konzentrationen unter allen einbezogenen Bedingungen. Statistische Analyse mittels linearem gemischten Modell ergab einen signifikanten Effekt des vokalen Aspekts auf Oxytocin- und Testosteronwerte, der zeigte, dass es beim Singen zu einer geringeren Abnahme dieser Hormone kam. Die Oxytocin-Veränderungen waren zudem bei den männlichen Teilnehmern ausgeprägter. Die Herzfrequenz konnte nicht mit Veränderungen in den Hormonkonzentrationen oder der Stimmung in

Verbindung gebracht werden, war jedoch während des Sololesens am niedrigsten. Insgesamt, verbesserte das Chorsingen die Laune der Teilnehmerinnen am meisten und führte außerdem zu einem erhöhten Identifikationsgefühl mit der Gruppe. Die Ergebnisse dieser Studie demonstrieren die positiven Auswirkungen des Singens auf soziale und emotionale Funktionen, und geben einen Einblick in die möglichen hormonellen Mechanismen, die dafür verantwortlich sein könnten.