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The Neurochemistry of Group Singing: Bonding and Oxytocin

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THE NEUROCHEMISTRY OF GROUP SINGING: BONDING AND OXYTOCIN

by

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The purpose of this study was to examine the neurochemical correlates of group vocal improvisation and to determine the feasibility of the research methods. One group of four participants sang together in two conditions: pre-composed and improvised. Concentrations of plasma oxytocin and adrenocorticotropic hormone (ACTH) were measured before and after each singing condition to assess levels of hormones associated with social affiliation, engagement and arousal. Successful implementation of the methodology, including recruitment, data collection, and sample analysis, served as the primary outcome of this study. ACTH concentrations decreased in both conditions, and significantly so in the pre-composed singing condition. Mean plasma oxytocin levels increased only in response to improvised singing, with no significant difference between improvised and pre-composed singing conditions. The results suggest that group singing may reduce stress and arousal, as measured by ACTH. Due to the small sample size, the effects of group singing on oxytocin are less clear. Social affiliation may be better facilitated in improvisatory experiences than pre-composed group singing, as measured by plasma oxytocin.

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Jason Keeler

TABLE OF CONTENTS

Table of Contents – Continued

Table of Contents – Continued

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

Statement of the problem

Music is used in healthcare to promote physical and psychological well-being. As clinical applications of music continue to expand, there is a growing need to understand the biological mechanisms by which music influences health. The social effects of music are of particular interest. Music is often used in clinical situations to build rapport, promote emotional expression, and enhance social skills. Board certified music therapists design and implement interventions to meet the social and health needs of their patients and clients. People often report a feeling of unity when they sing together, however, little is known about the neurobiological processes that facilitate that experience. Oxytocin is a hormone that promotes social bonding and trust in humans (Kosfeld et al., 2005). It is speculated that this hormone is also responsible for the social effects of music, however, this remains to be investigated (Chanda & Levitin, 2013). Adrenocorticotropic hormone (ACTH) is secreted in response to stress and mediates levels of arousal and engagement (Herman, 2012; Sandman et al., 1975; Lin et al., 2013). Examination of these hormones may help to understand how group singing creates feelings of connectedness and social bonding.

Rationale for research

As previously identified, research examining the neurochemistry of music

perception and production is still in its infancy. While only a handful of studies have examined neurobiological mechanism of music production, current behavioral findings indicate group music experiences, such as singing, can promote trust, social bonding, and well-being (Fancourt et al., 2014; Kreutz, 2014; Kreutz et al., 2004). Examining potential biological mechanisms underlying group singing may confirm previous behavioral findings and contribute to a growing body of evidenced-based research in music therapy. The cumulative effect of such studies is to promote access to care for patients and communities, as third-party reimbursement becomes more accessible with stronger research supporting the effectiveness of music therapy treatment. The goal of this study was to generate initial neurochemical data on group vocal improvisation, and to assess the feasibility of the research methods. As a basic scientific study, the findings from this project may inform future researchers examining clinical populations.

Research questions

Research Question 1

> Does pre-composed group singing elicit neurochemical changes associated with trust and attention, as measured by plasma oxytocin and ACTH?

Research Question 2

> Does improvised group singing elicit neurochemical changes associated with trust and attention, as measured plasma oxytocin and ACTH?

Research Question 3

> Will there be a significant difference in plasma concentrations of oxytocin and ACTH between pre-composed and improvised group singing?

Definitions of terms

Throughout this manuscript, the pre-composed singing condition will be henceforth referred to as the "standard" singing condition. The standard singing condition is operationally defined as a group vocal performance of the music as it was originally written, without further embellishment or improvisation. The improvised music condition is operationally defined as a group vocal performance that follows the syntactical (harmonic) structure of the pre-composed song, with significant embellishment and improvisation of the melody. In addition, the improvised music condition consists of improvised musical phrases exchanged between singers.

Summary

Previous behavioral findings and a handful of studies examining biological outcomes indicate that singing may promote health and well-being (Anshel& Kipper, 1988; Clift & Morrison, 2011; Welch et al. 2014). Research examining the neurochemistry of singing is warranted to further understand the effects of singing on health. Oxytocin may, in part, mediate the social and health benefits of group singing, while ACTH may in part facilitate attention and engagement. This appears to be the first study examining the neurochemistry of vocal improvisation. A feasibility study is warranted to identify effective research methods and generate initial hormonal data. The results of this study may inform larger studies and contribute to a growing body of research on the biological mechanisms underlying music production.

CHAPTER II

REVIEW OF LITERATURE

Music and social bonding

People often report a feeling of connectedness during music experiences, either as a listener or a performer. Audience members frequently share this sense of cohesion through a commitment to the music (Pitts, 2004). Little is known, however, about the neurochemical processes that facilitate social bonding during group music experiences. Over the past two decades, neuroscience research in music has relied heavily on neuroimaging, mapping regions of the brain active during music production and perception. It is only more recently that the neurochemical responses to music have been investigated. Chanda and Levitin (2013) review the chemical and biological effects of music and express a strong need for further research. Current evidence suggests that music's effects on health and well-being may be modulated through engagement of neurochemical systems (Chanda & Levitin, 2013; Fancourt et al., 2014). In particular, group singing has demonstrated positive effects on emotional states and biological outcomes, implicating the neuroendocrine system as a potential underlying mechanism (Fancourt et al., 2015; Kreutz, 2014; Kreutz et al., 2004). The neuropeptide oxytocin may in part be responsible for the social and health benefits of music, while adrenocorticotropic hormone (ACTH) may mediate the engagement and arousal effects of music (Chanda & Levitin, 2013; Kreutz, 2014). These physiological processes may consequently influence the subjective experience of social connection during music experiences.

Oxytocin

Oxytocin is a neuropeptide produced by large neuroendocrine cells of the supraoptic and paraventricular nuclei of the hypothalamus. Oxytocin is transported from the large neuroendocrine cells to the posterior lobe of the pituitary gland, where it is subsequently released into the bloodstream as a hormone. The paraventricular nucleus (PVN), where oxytocin synthesis is most concentrated, coordinates signals from the brain in response to stress and controls the hypothalamic-pituitary-adrenal (HPA) axis (Herman, 2012). Neurons in the PVN release corticotropin releasing-factor (CRF), which promotes the secretion of adrenocorticotropic hormone (ACTH) into peripheral circulation. ACTH is a neurohormone that stimulates the synthesis and release of glucocorticoids, such as cortisol, from the adrenal gland (Grossman et al., 1982). Oxytocin is colocalized with stress hormones in the PVN and has suppressive effects on the HPA axis, including ACTH (Carter, 2014; Gibbs, 1986; Windle et al., 2004).

The word oxytocin is derived from the Greek words meaning "quick birth". In humans, functions of oxytocin were initially identified with maternal behaviors such as mother-infant bonding, breast-feeding, and uterine contractions (Takahashi et al., 2013). More recent findings reveal the broader scope of oxytocin in human social and emotional behaviors, with effects that are highly dependent on context and individual traits (Bartz et al., 2011). Oxytocin mediates social behavior (Heinrichs et al., 2009) and regulates stress and anxiety (Ditzen et al., 2009). Depending on the context and the individual, it is hypothesized that oxytocin may elicit positive or negative social emotions (Bartz et al., 2011). Under optimal circumstances, oxytocin increases trust (Kosfeld et al., 2005) and is associated with parents' social attachment to their children (Feldman et al., 2010). While

oxytocin production in humans was originally believed to increase only in response to direct physical contact, mothers' bonding with their infants demonstrated higher plasma oxytocin levels from vocalizations alone (Leslie et al., 2010). The extended period of nurturing facilitated by oxytocin, as well as its role in reproductive behavior and physiologic functions, indicate its importance in human social and intellectual development (Carter, 2014). Research linking social behaviors to positive health and disease outcomes implicates oxytocin as a primary physiologic mechanism (Uchino, 2006).

ACTH

ACTH may mediate the engagement and arousal effects of music (Chanda& Levitin, 2013). ACTH, which mediates attention (Sandman et al., 1975; Sandman et al., 1977) and distress (Mauri & Volpe, 1994), responds to various types of challenges or pain perceived in higher levels of the brain (Herman, 2012). While there appears to be a general consensus among studies that music listening enhances oxytocin synthesis, the role of ACTH is less clear. Preliminary evidence suggests that listening to stimulating music, such as techno, increases plasma ACTH while relaxing music reduces ACTH synthesis and circulation (Gerra et al., 1998). ACTH is examined in this study because it responds to stimuli in seconds (Weijnen & Slangen, 1970). The short duration of each singing condition in this study indicate that ACTH may be implicated in behaviors of arousal and attention. To date, no studies were found that examined both ACTH and oxytocin in active music production.

Social affiliation and engagement in music

A handful of studies have examined endogenous oxytocin during music production and perception. Postoperative patients listening to relaxing music through headphones demonstrated an increase in serum oxytocin and reported higher levels of relaxation compared to a control group with no music (Nilsson, 2009). Choral singing has been shown to increase salivary oxytocin and elicit positive emotional states (Kreutz, 2014; Kreutz et al., 2004). In professional and amateur singers, peripheral oxytocin increased after an individual 45 minute singing lesson, however, music parameters were not identified and non-musical interactions during the lesson may have influenced outcome measures (Grape et al., 2002). In that same study, amateur singers demonstrated a decrease in post-singing levels of cortisol, while professional singers showed the opposite trend, pointing toward higher levels of perceived stress and arousal in the professional singers. This trend is consistent with the recent findings of Fancourt et al. (2015), where low-stress singing without an audience reduced levels of salivary cortisol and cortisone, and high-stress singing in front of a large audience increased levels of both glucocorticoids. Depending on individual traits and context, it is possible that singing may be perceived as a stressful experience with corresponding biological responses. In general, singing appears to have potential benefits on psychological health and well-being, with additional indications of potential physical benefits (Clift et al., 2010). More research is needed, however, to identify the underlying mechanisms linking singing to physical and psychological health.

While there is little information on the neurochemical correlates of singing, previous research has demonstrated the effects of singing on behavioral and self-reported

outcomes. Group singing produced the highest scores on trust and cooperation compared to other group activities, as measured by a trust and dilemma game (Anshel& Kipper, 1988). In those with mental illness, singing has been found to increase mental health, well-being, and social skills (Clift & Morrison, 2011). Children's sense of inclusion and belonging with their peers was positively correlated with their singing abilities in a longitudinal study on the social impact of music (Welch et al. 2014). This falls in line with the theory that music has evolved as a means of social bonding, with evolutionary roots in parent-infant attachment (Freeman, 1998). Therefore, the hypothesis that oxytocin plays a substantial role in the social and health benefits of music appears to be supported by previous behavioral findings.

Summary

Music experiences may promote social bonding and well-being, as demonstrated in previous studies. Musicians and audience members often report a sense of cohesion through shared music experiences. The neuropeptide, oxytocin, may in part be responsible for the social and health benefits of music. ACTH may mediate engagement and arousal during music production. The primary purpose of this study was to evaluate the design and methodology. In a recent review, LaGasse (2013) highlights the importance of pilot and feasibility studies in music therapy, especially in previously unstudied areas. Preliminary findings from this study may inform future researchers on the feasibility of neurochemical data collection in vocal music production. The results may also contribute to the growing body of evidence examining the neurobiological effects of music.

CHAPTER III

METHOD

Participants: Recruitment, enrollment, and demographics

Jazz vocalists at Western Michigan University (WMU) were recruited to explore the neurochemical correlates of vocal improvisation within a group context. Vocal quartets are common in jazz music, and therefore, the group structure was familiar to participants and limited extraneous challenges in a controlled setting. The director of WMU'S School of Music vocal jazz ensembles, Professor Greg Jasperse, was provided with flyers to disseminate to his vocal jazz students (Appendix A). Professor Jasperse provided hard copies of the recruitment flyer at the end of a rehearsal and read from a recruitment script (Appendix B). Potential participants were provided with the student investigator's e-mail address and phone number via the flyer. The investigator responded via e-mail or phone, with a scripted response (Appendix C) depending on the method of contact from the potential participant (e-mail or phone).

The recruitment goal was six participants: four subjects and two alternate subjects in case of no-shows. Out of 32 potential participants, five participants responded and were invited to individually review and sign the consent form (Appendix D) prior to the experiment. Inclusion criteria were the following: Jazz vocalists, students at Western Michigan University, and over 18 years of age. Student status and vocal jazz experience was verified by associate professor of music and director of vocal jazz ensembles, Greg Jasperse, prior to handing out the flyers. Exclusion criteria, based on Kosfeld et al. (2005)

were the following: Medical or psychiatric illness, smoking more than 15 cigarettes per day, drug or alcohol abuse, weighing less than 110 lbs, bleeding disorders (e.g. hemophilia), and pregnancy. Exclusion criteria was determined during the consent signing on a self-report basis. Participants were asked to abstain from food and drink (other than water) two hours before the experiment, and from smoking, caffeine, and alcohol 24 hours before the experiment. All five participants arrived the day of the experiment, with one participant serving as an alternate. Participants, including the alternate, were compensated with a $50 amazon.com gift card.

Research design

A two-way design using repeated measures was used to compare the effects of pre-composed versus improvised singing on plasma ACTH and oxytocin, both within subjects and between conditions. Each participant served as their own control and sang in both conditions (SP and IP).

Standard and improvised performance conditions

Musical criteria were identified in collaboration with the university's School of Music vocal jazz director, who was familiar with the participants' skill level and repertoire. The jazz standard "Centerpiece" (Edison & Hendricks, 1958) served as the musical content for both the standard and improvised conditions. The vocal jazz director created two vocal quartet arrangements of the piece, one for the standard performance condition (SP) and one for the improvised performance condition (IP). The SP arrangement was sung as written, with no improvisation or embellishments. The IP

arrangement began with the unison singing of the original melody and then allowed time for each participant within the group to improvise over the basic harmonic structure of the original song. To familiarize participants with the song and structure of each condition, a practice period of approximately five minutes was facilitated by the vocal jazz director prior to each performance.

Outcome measures

Outcome measures included pre- and post-test plasma levels of oxytocin and ACTH in each condition. Whole blood samples were obtained before and after singing in each performance. Oxytocin and ACTH concentrations were then determined using enzyme-linked immunosorbent assays kits produced by Enzo Life Sciences, Inc. (Farmingdale, NY, USA). The oxytocin kit has been previously validated for human plasma using various methods, including mass spectrometry (Carter et al., 2007). Sensitivity for oxytocin and ACTH was 15.0 pg/mL and .46 pg/mL, respectively. All samples were run in duplicate. Oxytocin plasma samples were diluted 1:8 with assay buffer and run unextracted. Both assays were run according to manufacturer instructions. Due to the small sample size, only one plate was needed to analyze each neuropeptide. The intra-assay coefficients of variations for ACTH and oxytocin were 15% and 9%, respectively. All tests were performed in collaboration with the Department of Biological Sciences at Western Michigan University.

There is controversy surrounding the measurement of oxytocin on unextracted samples. In human fluids, there may be interference from various proteins and other substances leading to unreliable measurements (McCullough et al., 2013). However, it is

also argued that extraction protocols may underestimate peripheral oxytocin concentrations, as a majority of oxytocin is lost during the extraction process (Martin and Carter, 2013). To avoid precipitation of oxytocin in the blood, samples were run unextracted using a previously validated, sensitive, and specific commercially-available kit.

Procedure

Participants (n=4) met at 4:00pm and formed one group together (a vocal quartet). At the beginning of the experiment, the consent form was reviewed and participants received a brief overview of the procedures. Participants were instructed to avoid physical contact during the experiment. SP pre-test blood draws were then conducted for two participants at a time in a separate room. The remaining two participants received the SP pre-test blood draw after each previous participant was finished. Given the labile nature of oxytocin and ACTH, two phlebotomists were used to expedite the blood collection process and minimize potential protein breakdown after whole blood was placed on ice. Following the SP pre-test blood draws, the vocal jazz director provided participants with brief musical instructions lasting approximately 5 minutes. Participants were instructed to sing their respective part of the music as it was written, without any embellishment or improvisation. Immediately following the instructional period, participants performed the standard piece together as it was written, with accompaniment provided on the piano by the aforementioned vocal jazz director. Immediately following the standard performance, which lasted 5 minutes and 38 seconds, participants were called in pairs to the separate room where individual post-test blood draws were

conducted. All participants confirmed that they were able to proceed without ill effects from the blood draws and were then escorted individually to nearby but isolated rooms for the 30-minute washout period. Following the 30-minute rest period, individual pre-test blood draws for the improvised performance were conducted. Following the same format as the SP condition, participants received 5 minutes of instructions prior to the improvised performance. Participants performed the improvised piece together with extensive embellishment and improvisation. The duration of the improvised performance was 6 minutes and 1 second. Immediately after the improvised performance, individual blood draws were conducted and participants were given a de-briefing. During that time, participants were provided with follow up information and thanked for their time.

Phlebotomy and sample storage

Phlebotomy procedures were identified in consultation with two trained phlebotomists, Brandy King and Jessica Toth. 6 ml of blood was drawn from the antecubital vein (located on the medial surface of the nondominant arm) into a chilled 6 ml EDTA lavender top tube containing 5.0 mg EDTA (an anti-coagulant) and 2.500 KIU aprotonin (a protease inhibitor to prevent degradation of the blood). A sterile field was maintained using a Vacutainer blood draw kit, unless the veins were determined to be ill suited for this method by the phlebotomist. In the event that was determined, either an injection needle or a push button (‘butterfly’) with syringe was used. 6 ml of whole blood yielded approximately 2 ml plasma after centrifugation. Approximately .2 ml of plasma was needed to analyze oxytocin samples in duplicate, while approximately 0.4 ml of plasma was needed to analyze ACTH in duplicate. Given the labile nature of each

hormone and the high-precision needed for each assay, the extra 1.4 ml of plasma accounted for any variation in plasma yielded from whole blood samples and potential mistakes made during the ELISA procedure.

The blood samples were labeled by the phlebotomist with the participant's confidential identification number and the experimental condition during which the blood was drawn (Pre SP, Post SP, Pre IP, or Post IP). For example, blood drawn from the participant with confidential identification number 3 and following the improvised performance was be labeled "3 Post IP". The labeled blood sample was immediately stored by the phlebotomist in an insulated and secured transport container packed with crushed ice. Samples were then transported from the Dalton Center to Dr. John Spitsbergen's laboratory in room # 3052 Haenicke Hall, where it was received by two research assistants.

Blood samples received at the lab were immediately centrifuged at 1500 rpm for 12 minutes at 4°C by the research assistants in biology in Dr. Spitsbergen's lab. The research assistants wore safety glasses, latex gloves, and a disposable mask (or a face shield) while handling blood samples and disposing of contaminated products. Immediately after the blood was centrifuged, plasma was withdrawn and placed into 2 ml microtubes with screw caps by the research assistants. Plasma was aliquoted in .250 ml amounts into each microtube to avoid repeated freeze-thaw cycles and to accommodate the amount of plasma needed for each ELISA test. Each microtube was labeled with a code indicating the participant number and the experimental condition. For example, "1A" indicated participant number 1 and pre-test condition 1 (standard singing), and "1B" indicated participant 1 and post-test condition 1. "2C" indicated participant number

2 and pre-test condition 2 (improvised singing), where as “2D” indicated participant number 2 and post-test condition 2.

Immediately after plasma was aliquoted into microbutubes, samples were placed in a labeled box and into a -70˚C freezer to await analysis. The lavender top tubes that previously contained whole blood were decontaminated with bleach and placed in biohazard bags provided by WMU’s Environmental Health and Safety department (see the following step and the appendices for detailed safety and disposal procedures). Proper disposal and decontamination procedures were identified in consultation with Clara Davis and Lu DeBoef of WMU’s Environmental Health and Safety department. Please see the Standard Operating Procedure and blood collection supplement in the appendices for detailed disposal and decontamination procedures. Proper disposal and decontamination procedures were followed during all stages of the experiment (collection, storage, and analysis). Prior to the experiment, disposal, safety, and decontamination materials were delivered to the B.R.A.I.N. lab by the Environmental Health and Safety department. The materials were stored in the locked B.R.A.I.N. lab (Rm 2019, Health and Human Services Building) until the start of the experiment. At the start of the experiment, all disposal and decontamination materials were in place and readily available at the site of data collection (the music therapy clinic) and data storage and analysis (Dr. Spitsbergen’s lab). The phlebotomists brought their own supplies to the music therapy clinic for blood collection procedures. At the end of data collection and analysis, all blood-related products stored in appropriate biohazard containers were picked up by the WMU Environmental Health and Safety department for proper disposal.

ELISA procedures

Each assay was run according to the manufacturer's procedures. Reagants were brought to room temperature at least 30 minutes prior to each analysis. Samples were removed from the deep freezer in Dr. Spitsbergen's lab and thawed immediately prior to each analysis. All standards and samples were run in duplicate. Unused wells were stored in a sealed pouch at 4°C. To reduce variability, all pipetting was performed by research assistant Daniel Waters. Standards were pipetted into appropriate wells followed by the samples. Conjugates and antibodies were then added into each well, followed by an incubation period ranging from 4 hours (ACTH ELISA) to 24 hours (oxytocin ELISA). Following incubation, plates were emptied, washed, and dried. Substrate was added to facilitate the enzyme reaction, followed by a stop solution to generate a yellow color in each well. In the oxytocin ELISA, the intensity of the yellow color was inversely proportional to the plasma concentration. In the ACTH ELISA, the intensity of the yellow color was directly proportional to the concentrations of ACTH present in the samples and standards. The optical density of each well was measured by a microplate reader in Dr. Spitsbergen's lab. Optical densities were converted into plasma hormone concentrations utilizing a software program provided by the ELISA manufacturers, called AssayBlaster.

Analysis of the data

Given the small sample size (n=4), this study was not sufficiently powered for statistical analyses, however, statistical tests were performed to demonstrate potential analyses for future studies with larger samples. Descriptive and parametric statistical

analyses were performed using SPSS (IBM) version 22. A paired *t*-test was conducted to compare the difference between pre-post levels of each hormone in both conditions.

CHAPTER IV

RESULTS

Oxytocin and ACTH

Descriptive data for oxytocin and ACTH collected at each time point are presented in Table 1. Both the standard and improvised conditions revealed a mean decrease in plasma ACTH after participants sang together. The change in ACTH in the SP condition pre-to-post test ($p < .05$) was 21% greater than the pre-to-post test ACTH outcomes for the IP condition ($p > .05$). Figure 1 depicts individual changes in ACTH at each time point. Student's *t*-test of oxytocin concentrations in the SP condition revealed a mean decrease of 10 pg/mL, while the IP condition demonstrated a mean increase of 27 pg/mL. Figure 2 depicts individual changes in oxytocin concentrations across all time points. In figure 3, individual changes in oxytocin and ACTH are compared at each time point to reveal an inversely proportional trend in 3 out of 4 subjects.

Table 1

Mean Oxytocin and ACTH Concentrations

Variable	SP Pre-test			SP Post-test			IP Pre-test			IP Post-test		
(pg/mL)	M	Med	SD	M	Med	SD	M	Med	SD	M	Med	SD
ACTH	27.3	26.1	11.6	20.2	17.1	9.0	15.9	15.9	1.1	15.2	16.1	5.3
OT	201.8	186.3	104.2	191.5	168.8	94.5	184.6	157.9	74.2	212.2	183.1	111.4

Note. Descriptive statistics of neurochemical variables, adrenocorticotropic hormone (ACTH) and oxytocin, at each time point in the standard (SP) and improvised (IP) conditions (n=4).

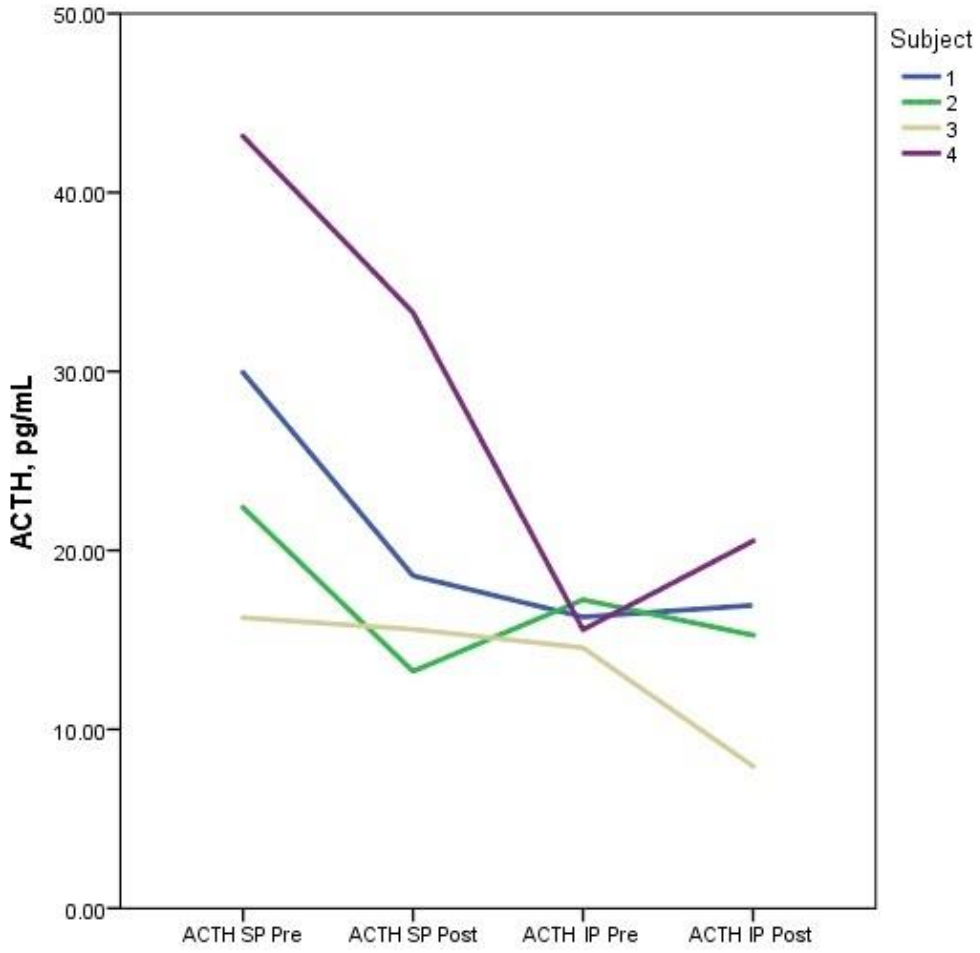

Figure 1. Individual ACTH Concentrations Before and After Each Singing Condition

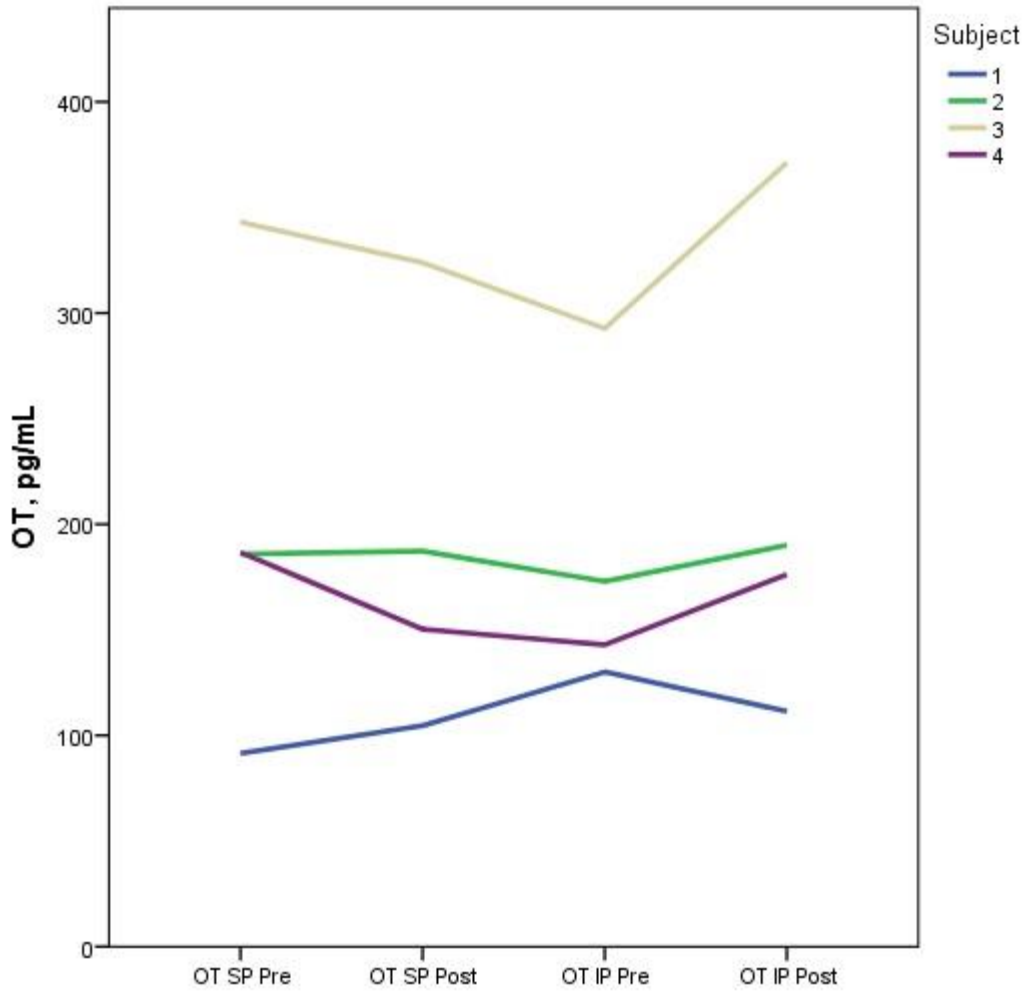

Figure 2. Individual Oxytocin Concentrations Before and After Each Singing Condition

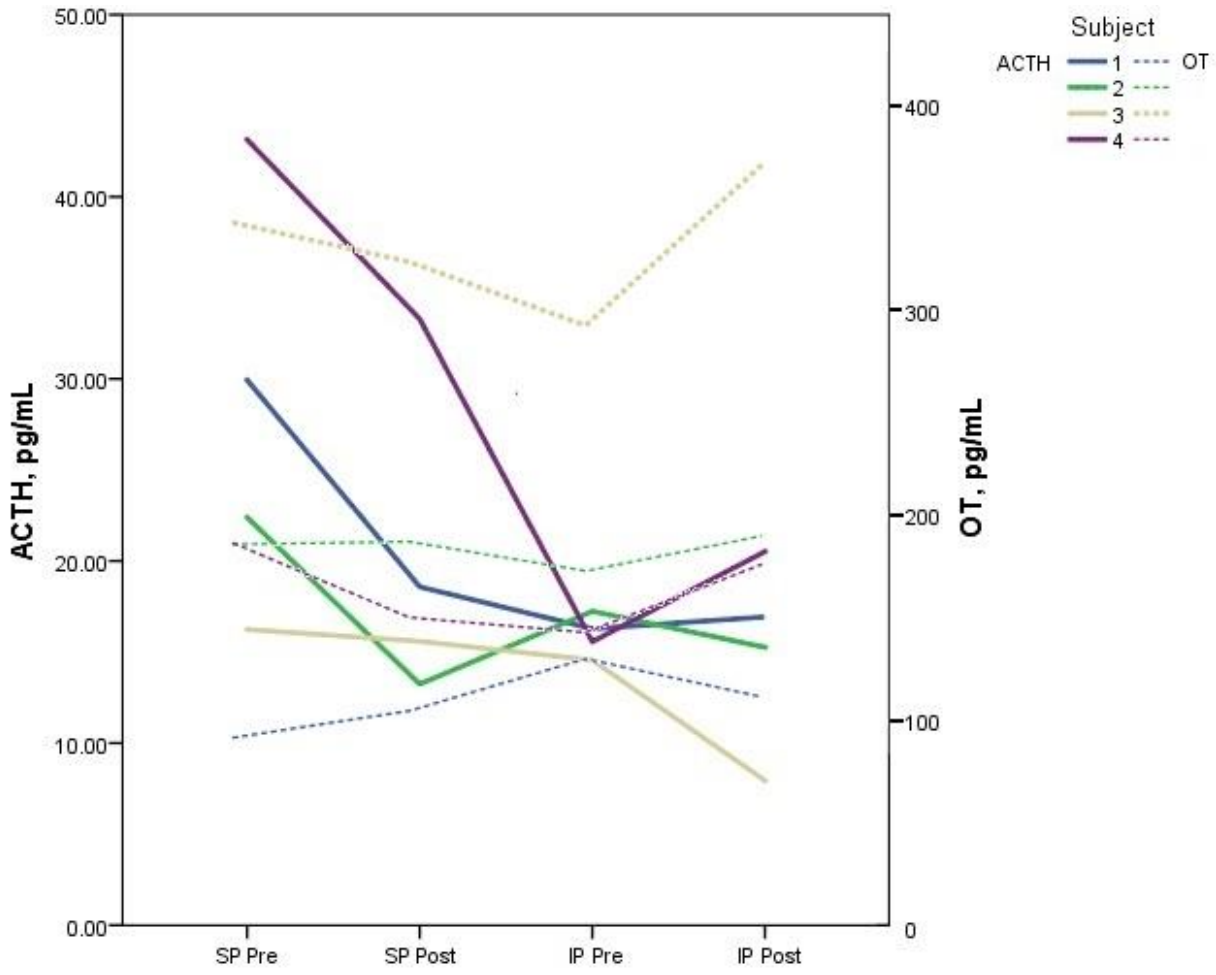

Figure 3. Individual Oxytocin and ACTH Concentration Comparisons Between Singing Conditions

Research question 1

Does pre-composed group singing elicit neurochemical changes associated with trust and attention, as measured by plasma oxytocin and ACTH?

There was a significant decrease in plasma ACTH following pre-composed group singing. This may indicate a decrease in stress and arousal experienced by participants following singing. There was no significant difference in mean oxytocin levels following pre-composed group singing. This should be interpreted

with caution, however, due to the small sample size and high variability of plasma oxytocin levels between participants.

Research question 2

Does improvised group singing elicit neurochemical changes associated with trust and attention, as measured by plasma oxytocin and ACTH?

There was no significant change in neurochemical measures following improvised group singing. Descriptive statistics revealed a mean decrease in ACTH following improvised group singing, and a mean increase in oxytocin. Again, those results should be interpreted with caution due to the small sample size.

Research question 3

Will there be a significant difference in plasma concentrations of oxytocin and ACTH between pre-composed and improvised group singing?

There was no significant difference in plasma concentrations of oxytocin and ACTH between pre-composed and improvised group singing. Improvised singing resulted in a mean increase in plasma oxytocin, while pre-composed singing resulted in a mean decrease in oxytocin. Mean ACTH concentrations decreased in both conditions.

CHAPTER V

DISCUSSION

Feasibility of experimental procedures

The primary purpose of this study was to evaluate the feasibility of experimental research procedures that were designed to investigate the hormonal changes associated with group singing. The successful implementation of procedures and data collection indicates the feasibility of the methods employed in this study, which may help future researchers in exploring biological mechanisms underlying music production.

Oxytocin and ACTH

It was hypothesized that group singing in both conditions would decrease stress and arousal, as measured by ACTH, and increase social bonding, as measured by oxytocin. Despite the small sample size, there was a significant decrease in pre-to-post levels of ACTH during the standard singing performance. This is consistent with previous literature demonstrating the positive effects of music on stress and the immune system (Bittman et al., 2001; Chanda& Levitin, 2013). Singing has been shown to reduce cortisol levels depending on context and individual traits, and is generally associated with psychological health and well-being (Grape et al., 2002; Clift et al., 2010; Fancourt et al., 2015).This is the first time that ACTH has been examined during active music production, as opposed to music listening, and the results appear to be supported by previous findings. The improvised singing performance demonstrated a minor decrease in

ACTH from pre-to-post levels, which perhaps may be attributed to low concentrations observed at the IP pre-test. This may have been caused by carry-over effects from the first condition (see recommendations for future research).

As expected, mean concentrations of oxytocin increased during the improvised condition. Informal observations revealed that vocal improvisation naturally elicited behaviors conducive to social bonding, such as listening, responding, spontaneous communication, eye contact, and cooperation. Surprisingly, the standard performance demonstrated a mean decrease in oxytocin concentrations. This should be interpreted with caution due to the small sample size and influence of individual traits on plasma oxytocin levels (Bartz et al., 2011). Studies with a larger sample size have demonstrated significant increases in peripheral oxytocin after choral singing and individual singing lessons (Grape et al., 2002; Kreutz, 2014). The high variability of oxytocin concentrations observed among participants in the present study can be addressed through a larger sample size. A sufficiently powered study using the same design as the current study ($1 - \beta = .80$, effect size $> .25$, alpha $< .05$) would require 82 participants based on our data.

Limitations

Several limitations need to be considered when interpreting the results of this study. The small sample size provides initial data, however, it does not provide enough power to make strong statistical inferences. Prior to conducting the study, participants were informed that the researchers were investigating differences between standard and

improvised vocal performance. This knowledge may have influenced responses either during or following each task.

Recommendations for future research

Results from this study generated initial, hormonal data on group vocal improvisation. As previously stated, a primary recommendation is implementing the current methodology with a larger sample size. To avoid potential carry over effects, a longer washout period between conditions is recommended. Although previous studies indicated only a short washout period was necessary, mean plasma ACTH at baseline was significantly lower in the second (IP) condition. This may have also been attributed to an order effect, as all participants sang in the IP condition following the SP condition. A repeated measures design with counterbalancing may control for any order effect observed in this study. A washout period at the beginning of the experiment may also reduce variations in neuropeptide levels caused by pre-experimental stimuli. Given the recent findings of Fancourt et al. (2015), a potential extension of this study may also include a rehearsal period and self-report data to evaluate the level of stress experienced by participants during standard and improvised singing. In the present study, the significant decrease in ACTH during the standard performance may indicate a low-stress condition experienced by participants. The stress level of the improvised performance is less clear, however, the upward trend of ACTH and informal statement from one of the participants may perhaps indicate a higher level of stress experienced while improvising. The structure of each performance more closely resembled a practice or rehearsal, as

evidenced by Professor Jaspers' guidance at the beginning of each performance and the absence of an audience.

During this study, the researchers informally noted differences in participants' behavioral tendencies between conditions. For example, a higher frequency of social interaction cues, such as eye contact, were observed during the improvisation task. Behavioral analyses combined with 7-8 formal interview questions of the participants' subjective experience would provide greater insight on social affiliation in standard and improvised group singing. A further extension of this study may consider examining the neurochemical correlates of trust and social affiliation during group instrumental improvisation. Music therapists often employ instrumental improvisation in clinical practice to achieve non-musical goals. Clinical improvisation offers an opportunity for self-expression and emotional processing that may not otherwise be available to individuals with social deficits or mental disorders. It remains to be explained, however, how music exerts its effects on trust and social bonding. Oxytocin may be a potential mechanism underlying the social effects of clinical improvisation, and further investigation is warranted. Vasopressin, a similar neuropeptide, may also underlie the social effects of music and could be an important variable to examine in future studies. It is further recommended that a randomized control design be utilized to allow for causal inferences. Lack of randomization and a control group in the current study does not support the notion that singing causes changes in neurochemical correlates, but rather is associated with those changes. An ABA design may also be useful when participants and resources are limited, such as in the current study.

Appendix A

Recruitment Flyer

SING FOR SCIENCE!

A GRADUATE RESEARCH STUDY

You are invited to participate in a research study exploring singing and social bonding. We are interested in the biology of social connectedness experienced during singing.

Who is eligible?

- Must be a jazz vocalist and student at WMU
- Must be over 18 years old

What will you be asked to do?

- Sing and improvise in a vocal jazz quartet
- Have blood drawn and complete two questionnaires
- Approximately 2 hour time commitment

Compensation: Each participant will receive a $50 Amazon.com gift card

To learn more, contact one of the student investigators of this study, Jason Keeler or Brittany Neuser, at jason.r.keeler@wmich.edu, brittany.l.neuser@wmich.edu, or (269) 387-8841.

Western Michigan University

BRAIN Lab

Appendix B

Recruitment Script

Script presented by vocal jazz instructor, Greg Jasperse, to vocal jazz students:

"Our colleagues in Music Therapy are conducting a study that will investigate the neurochemistry of singing, including improvising vocally. If you are interested in participating or finding out more about the study, their contact information is on the flyer and you are invited to call or e-mail some time in the next week."

Appendix C

E-mail script in response to potential participants:

Dear [insert name],

Thank you for your interest in this study. My name is [Jason Keeler or Brittany Neuser] and I am writing on behalf of myself and [Jason Keeler or Brittany Neuser]. We are both graduate students in Music therapy and we are conducting a study that will serve as our Master's theses. The purpose of this study is to examine the biology of singing, in particular, we want to understand the neurochemistry involved with the feelings of bonding and connectedness people often report after singing together. You will be asked to sing and improvise with others in a vocal jazz quartet. Should you choose to participate, you will be asked to sing twice in a single time period. You will sing a standard performance of a song as the music was originally written, and an improvised performance using the same song. Afterward, you will be asked to complete a brief questionnaire. The total time commitment for this study is approximately 120 minutes.

Your participation is voluntary. Due to the nature of this study, participants that meet any of the following criteria are not eligible to participate: Significant medical or psychiatric illness, smoking more than 15 cigarettes per day, drug or alcohol abuse, pregnancy, bleeding disorders, and weighing less than 110 lbs. Please use your best judgment in determining your eligibility for this study.

Should you be interested in participating, please respond to this e-mail at your earliest convenience.

Sincerely,

Jason Keeler/Brittany Neuser

Appendix D

HSIRB approval letter

WESTERN MICHIGAN UNIVERSITY

Human Subjects Institutional Review Board

Date: March 20, 2015

To: Ed Roth, Principal Investigator
Jason Keeler, Student Investigator for Thesis
Brittany Neuser, Student Investigator for Thesis

From: Amy Naugle, Ph.D., Chair

Re: HSIRB Project Number 14-11-23

This letter will serve as confirmation that the changes to your research project titled "Neurochemistry of Singing: Social Bonding and Oxytocin" requested in your memo received March 20, 2015 (to increase number of participants from four to six) have been approved by the Human Subjects Institutional Review Board.

The conditions and the duration of this approval are specified in the Policies of Western Michigan University.

Please note that you may **only** conduct this research exactly in the form it was approved. You must seek specific board approval for any changes in this project. You must also seek reapproval if the project extends beyond the termination date noted below. In addition if there are any unanticipated adverse reactions or unanticipated events associated with the conduct of this research, you should immediately suspend the project and contact the Chair of the HSIRB for consultation.

The Board wishes you success in the pursuit of your research goals.

Approval Termination: December 21, 2015

251 W. Walwood Hall, Kalamazoo, MI 49008-5456
PHONE: (269) 387-8293 FAX: (269) 387-8276

Appendix E

HSIRB consent form

Western Michigan University
School of Music

Principal Investigator: Edward Roth, MM, MT-BC
Student Investigator: Jason Keeler, MT-BC
Student Investigator: Brittany Neuser, MT-BC
Title of Study: Neurochemistry of Singing: Social Bonding and Oxytocin

You have been invited to participate in a research project titled "The neurochemistry of singing: Oxytocin and social bonding." This project will serve as Jason Keeler and Brittany Neuser's theses for the requirements of the Master's of Music. This consent document will explain the purpose of this research project and will go over all of the time commitments, the procedures used in the study, and the risks and benefits of participating in this research project. Please read this consent form carefully and completely and please ask any questions if you need more clarification.

What are we trying to find out in this study?
The purpose of the present study is to examine the effects of vocal improvisation on biological measures of trust and social bonding. People often report feelings of unity or connectedness during group music experiences, however, little is known about the neurotransmission processes that facilitate these experiences. This study will examine oxytocin and adrenocorticotropic hormone, which are associated with feelings of social bonding often experienced during group singing. Also, musicians often report experiencing a mental state known as "flow" while performing music. You will be asked to complete a written survey that will help us assess the level of flow you may have experienced while singing during this experiment.

Who can participate in this study?
To be able to participate in this research project, you must meet the following criteria:

- You must be over the age of 18.
- You must not have a significant medical or psychiatric illness, smoke more than 15 cigarettes per day, abuse drugs or alcohol, have any bleeding disorders (e.g. hemophilia), weigh less than 110 lbs, or be pregnant.
- Participants must be jazz vocalists with the ability to improvise vocally.

Where will this study take place?
Study procedures and data collection will take place in the music therapy clinic, room 3025, located on the third level of the Dalton Center on Western Michigan University's main campus.

What is the time commitment for participating in this study?

The total time commitment for this study is approximately two hours.

- Read and sign consent form prior to the day of study procedures: 10 minutes
- Study procedures: 110 minutes

What will you be asked to do if you choose to participate in this study?

During this study, you will be asked to sing in a jazz quartet with three other participants. You will be asked to sing two times, once from a score as the music was originally written and a second time with extensive improvisation using the same score. Prior to, and immediately following each singing condition, you will be asked to have your blood drawn by a trained phlebotomist, for a total of four blood draws. You will be asked to fill out two surveys during this study.

The student investigator will call you 48 hours prior to the experiment to remind you about the time and location of the study.

When you arrive for the experiment, the student investigators will ask if you would like to have the study and all of its procedures, risks, and benefits explained to you again. You will be encouraged to ask any questions that you may have.

We will ask that you not smoke, drink any alcohol or take any caffeine the day before and the day of your visit for the experiment. We will also ask that you not eat or drink (other than water) two hours before the experiment. Also, we ask that you come to the Dalton Center wearing comfortable clothing that will allow the phlebotomist to draw blood from your arm.

What information is being measured during the study?

We will be taking measurements of two hormones found in blood: oxytocin and adrenocorticotropic hormone. In addition, we will be measuring your sense of "flow" through a written survey instrument.

What are the risks of participating in this study and how will these risks be minimized?

A risk of providing blood can include mild to moderate pain at the site of the needle puncture into the vein. Other risks could include temporary redness, minor bleeding, swelling and a bruise at the site of the needle puncture or, rarely, an infection. Ice will be available if minor swelling or bruising occurs. Should signs of an infection present, please seek consultation with a physician at Sindecuse Health Center, Bronson Hospital, or with your primary care physician. Some people feel dizzy or faint when blood is taken; however, most people do not experience any problems. These risks will be minimized by an experienced phlebotomist trained in proper blood draw and sterilization techniques. Snacks and water will also be available should you feel faint. Although possible, most people do not experience any problems given the limited amount of blood collected for this study's purposes.

As in all research, there may be unforeseen risks to the participant. If an accidental injury occurs, appropriate emergency measures will be taken; however, no compensation or additional treatment will be made available to you except as otherwise stated in this consent form.

What are the benefits of participating in this study?
There are no direct benefits to you from participating in this study. If you are interested, the investigators will provide you a copy of the results after the data have been analyzed and the study has been completed. You may be interested in learning about research and some of the laboratory procedures used in collecting research data.

Are there any costs associated with participating in this study?
There are no costs associated with participating in this study.

Is there any compensation for participating in this study?
Each participant will receive a $50 Amazon.com gift card.

Who will have access to the information collected during this study?
The principal investigator, student investigators, and committee members of this thesis project will have access to the information collected during the study. In order to maintain confidentiality, a random identification number will be assigned to you and used in place of your name. The original data will be retained in a locked cabinet in the locked office of the Principal Investigator in the School of Music at Western Michigan University for a minimum of three years after the completion of the study.

What if you want to stop participating in this study?
You can choose to stop participating in the study at anytime for any reason. You will not suffer any prejudice or penalty by your decision to stop your participation. You will experience NO consequences either academically or personally if you choose to withdraw from this study.

Should you have any questions prior to or during the study, you can contact one of the student investigators, Jason Keeler or Brittany Neuser, at 269-387-8841, or the primary investigator, Edward Roth at 269-387-5415. You may also contact the Chair, Human Subjects Institutional Review Board at 269-387-8293 or the Vice President for Research at 269-387-8298 if questions arise during the course of the study.

This consent document has been approved for use for one year by the Human Subjects Institutional Review Board (HSIRB) as indicated by the stamped date and signature of the board chair in the upper right corner. Do not participate in this study if the stamped date is older than one year.

I have read this informed consent. The risks and benefits have been explained to me. I agree to take part in this study.

_____

Please Print Your Name

_____ _____

Participant's signature Date

Appendix F

Standard operating procedure

Standard Operating Procedure

Subject: Prevention of Occupational Exposure to Bloodborne Pathogens such as HIV, Hepatitis B and other infectious diseases.

Department: Music

Date: 11/14/2014

Job Classifications:

Category A: Phlebotomists

Category B: All other positions.

Tasks:

1. Drawing blood for the analysis of oxytocin and adrenocorticotropic hormone.
2. Cleaning tasks involving contaminated areas and non-disposable instrumentation.
3. Centrifuging and storing plasma samples.
4. Analyzing samples using enzyme-linked immunoassays.
5. Disposing of leftover sample and contaminated equipment.

Equipment:

Building Equipment:

1. Blood draw facilities will be equipped with disposable latex or hypoallergenic gloves and disinfectant wipes.
2. Hand washing and eye washing facilities will be readily accessible to staff.
3. Containers or bags for biohazardous material will be available in the music therapy clinic (room 3025 in the Dalton Center) where the blood collection will take place, and in Dr. Spitsbergen's lab (room 3052 Haenicke Hall) where storage and analysis of the samples will occur. Biohazard containers or bags must be red or orange with the contrasting biohazard symbol and lettering. These containers and bags must be brought to the site of biohazard waste generation or clean up for disposal of material. Blood or other blood fluid is to be contained in the immediate area of biohazard waste generation or clean up and shall not be moved across the facility.
4. Main areas for consumer activities will each have a container of disinfectant wipes.

5. Red, puncture-resistant, leak proof, properly labeled sharps container will be available on-site for sharps such as vacutainer blood collection needles, syringes, eclipse injection needles, and push button collection needles.

Work Practice Controls:

1. All category A employees are offered the Hepatitis B vaccine upon hire. Staff may decline the vaccine, but must do so by signing a form letter indicating such, to be placed in their personnel file.
2. Latex or vinyl gloves will be worn for any task where there is any potential of exposure to blood or other potentially infectious material (i.e. body fluids, mucous membranes, skin with open areas or surfaces soiled with blood or body fluids). These tasks would include, but are not limited to, drawing blood for testing purposes, and cleaning up of blood, or other potentially infectious material. These tasks would also include the storage and analysis of blood samples.
3. After use, gloves and other disposable material such as wipes and bandages, will be discarded in a regular trash container, as long as they do not contain any blood.
4. Material contaminated with blood or other potentially infectious materials will be disposed of in a biohazard container that is brought to the site of the contamination.
5. Hands will be thoroughly washed with soap and water as soon as possible after removal of the gloves.
6. Hands must be cleaned with alcohol gel, if hand washing facilities are not available.
7. Gloves must be changed after each task that requires their use.
8. Blood spills will be cleaned up promptly with materials in the blood spills kit.

Exposure Incident:

1. If an individual experiences an exposure to blood or other potentially infectious material, he/she must immediately report to a supervisor (Professor Edward Roth) who will immediately send the individual to the Sindecuse Health Center or Bronson Hospital Emergency Room. The physician on duty will determine if an actual exposure has occurred.
2. An accident/injury 311 form must be completed by the supervisor for any exposure incident. The supervisor (Professor Edward Roth) must complete an Employee Injury WC-210 form.

Biohazard Waste Disposal:

1. WMU Environmental Health and Safety (387-5593) will be called to pick up the biohazard bags and sharps containers. Bags or containers are to be properly labeled or color coded as containing hazardous waste.
2. Biohazard container will be kept in a secured area such as locked closet or office until EHS can pick up.

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