Social Psychophysiology for Social and Personality Psychology

The SAGE Library of Methods in Social and Personality Psychology is a new series of books to provide students and researchers in these fields with an understanding of the methods and techniques essential to conducting cutting-edge research.

Each volume explains a specific topic and has been written by an active scholar (or scholars) with expertise in that particular methodological domain. Assuming no prior knowledge of the topic, the volumes are clear and accessible for all readers. In each volume, a topic is introduced, applications are discussed, and readers are led step by step through worked examples. In addition, advice about how to interpret and prepare results for publication is presented.

The Library should be particularly valuable for advanced students and academics who want to know more about how to use research methods and who want experience-based advice from leading scholars in social and personality psychology.

Published titles:
James J. Blascovich, Eric Vanman, Wendy Berry Mendes & Sally Dickerson, *Social Psychophysiology for Social and Personality Psychology*

R. Michael Furr, *Scale Construction and Psychometrics for Social and Personality Psychology*

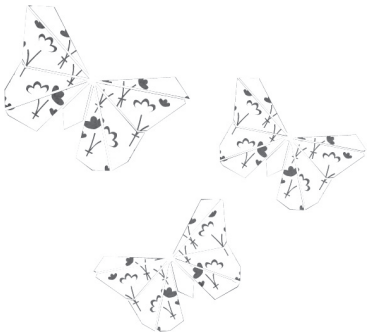
Rick H. Hoyle, *Structural Equation Modeling for Social and Personality Psychology*

John B. Nezlek, *Multilevel Modeling for Social and Personality Psychology*

Laurie A. Rudman, *Implicit Measures for Social and Personality Psychology*

Forthcoming titles:
John B. Nezlek, *Diary Methods for Social and Personality Psychology*

The SAGE Library in Social and Personality Psychology Methods



Social Psychophysiology for Social and Personality Psychology

Jim Blascovich,
Wendy Berry Mendes,
Eric Vanman, Sally Dickerson

SAGE

Los Angeles | London | New Delhi
Singapore | Washington DC

First published 2011

SAGE Publications Ltd
1 Oliver's Yard
55 City Road
London EC1Y 1SP

SAGE Publications Inc.
2455 Teller Road
Thousand Oaks, California 91320

SAGE Publications India Pvt Ltd
B 1/I 1 Mohan Cooperative Industrial Area
Mathura Road
New Delhi 110 044

SAGE Publications Asia-Pacific Pte Ltd
33 Pekin Street #02–01
Far East Square
Singapore 048763

Library of Congress Control Number: 0000000

British Library Cataloguing in Publication data

A catalogue record for this book is available from the British Library

ISBN 978–0-85702–405–3

Typeset by C&M Digitals (P) Ltd, Chennai, India
Printed in Great Britain by [to be supplied]
Printed on paper from sustainable resources

Contents

List of Figures and Tables

Figures

Tables

1

Introduction

The goal of this book is to provide methodological and technical information for social psychologists and other behavioral scientists who are considering using peripheral neurophysiological and endocrine measures of psychological constructs. This volume is neither exhaustive in terms of the entire range of candidate endocrine and peripheral neurophysiological systems nor even of the range of measures within the physiological systems described. Rather, it is meant to give investigators an informed basis for determining how to validly and valuably use endocrine and peripheral physiological indexes of social psychological constructs as measures in their empirical endeavors.

Advantages of Using Physiological Measures in Social Psychological Research

The major advantages of measuring endocrine and peripheral neurophysiological responses for social psychologists have been articulated in detail elsewhere (e.g., Blascovich, 2000) and include the ability to obtain responses continuously, covertly, and online. Assuming such measures or indexes are relatively strong inferentially (i.e., markers or invariants; see Figure 1.1), continuous measurement provides the temporal topology of a physiological response allowing for the recording of meaningful changes within experimental episodes. For example, the rise and fall slopes and times of a response may be more sensitive and, therefore, more meaningful than a simple means of a response. The fact that such measures are covert generally insures that research participants do not monitor and adjust their responses. This advantage enables researchers to index psychological states without either observing deliberative behaviors or asking participants to answer introspective questions about their feelings, intentions, and thoughts. Finally, the advantage that physiological measures are online means that researchers do not have to rely on inferences based on prospective or retrospective self-reports.

Adding to these advantages, physiological responses can be superior to explicit measures because they are generally more *sensitive*, *uncensored*, *prognostic*, and *mechanistic*. Such responses may provide more *sensitive* indicators of psychological

states than explicit indicators, such as self-reports, for at least two reasons. The main one is that physiological responses typically occur below conscious awareness and can be sensitive to changes in mental states that individuals are *unable* to report. That is, participants are generally unaware of the shifts in their physiology, let alone patterns of processes that reflect mental processes and, thus, are unable to consciously report them. However, precise measurements of the responses themselves are sensitive to these processes and consequently mark them even when explicit indexes do not. For example, heart rate and ventricular contractility data can be used to assess task engagement on the part of participants in studies involving performance (see Chapter 2).

Although there is much to learn if one wants to successfully incorporate psychophysiology into the methodological toolbox of social psychology, the advantages make it worth it. Using physiological measures adds to the research enterprise including theory development, testing, and application. For example, the Biopsychosocial Model of Challenge and Threat (Blascovich & Tomaka, 1996) rests on neuroendocrine theory developed on the basis of animal studies (Dienstbier, 1989) and validated in humans (e.g., Tomaka, Blascovich, Kelsey & Leitten, 1993), used to test threat-based stigma theory (e.g., Blascovich, Mendes, Hunter, Lickel & Kowai-Bell, 2001), and applied to performance prediction (e.g., Blascovich, Seery, Mugridge, Weisbuch & Norris, 2004). Some monetary costs are accrued, both in terms of acquisition and maintenance of equipment, though nothing like the costs of acquiring and using brain imaging technologies. So, it is fitting here to discuss some of the benefits of using psychophysiological measures.

In terms of measurement, autonomic responses typically provide data at a ratio level of measurement, whereas explicit measures such as self-reports are often ordinal within small, circumscribed ranges. Unlike Likert-type self-report scales that force a ceiling or floor on measurements, changes in physiological responses, for example, autonomic nervous system (ANS) reactivity measures, cover broad ranges of values so that physiological values with known endpoints indicate meaningful and valid differences in psychological states and processes.

For example, patterned changes in ANS cardiovascular responses, indexing psychological states of *challenge* versus *threat*, are a better predictor of a decision-making heuristic – anchoring and adjustment – than self-reported ones (Kassam, Koslov, & Mendes, 2009). In this study, individuals were randomly assigned to either a "challenge" or "threat" state. Even though self-reports and physiology converged on participants' assessments of the randomly assigned challenge or threat state, only the ANS responses mediated the link between the manipulated experimental condition and subsequent decision-making processes. More specifically, explicit appraisals estimated less than one-quarter of the explanatory power that cardiovascular changes did in predicting decision-making processes. These data are important because they suggest that ANS responses can be more sensitive indicators of psychological processes than explicit self-reported states.

Physiological responses generally provide an *uncensored* view of people's mental states because they can reveal psychological states that individuals may be *unwilling* to report. For social psychologists, attention to physiological changes has some parallels with law enforcement agencies' use of ANS responses for "lie detection" purposes – criminals often confess to a crime if they believe someone has insight into their "true" thoughts as revealed via ANS measures (though there is little evidence supporting their validity; Feinberg & Stern, 2003). The *bogus pipeline* (Jones & Sigall, 1971), for example, was introduced specifically for the purposes of coaxing individuals to self-report their *true* thoughts and feelings because such individuals were led to believe that their bodies would betray them by producing signals that they could not control. Although the bogus pipeline did not involve actual physiological measures, participants thought it did. Like so-called lie detection, the same effect on veridicality of self-reported responses obviously can accrue to research actually involving physiological responses.

There are also domains in which individuals might be unwilling to explicitly report their thoughts and feelings because of self-presentational issues. For example, in the last 10 to 15 years, the number of studies examining intergroup interactions using psychophysiological techniques has surged (e.g., Amodio, Harmon-Jones & Devine, 2003; Blascovich, Mendes, Hunter, Lickel & Kowai-Bell, 2001; Mendes, Blascovich, Lickel & Hunter, 2002; Mendes, Blascovich, Hunter, Lickel & Jost, 2007; Scheepers, 2009). This increase is likely due to the possibility of demand characteristics inherent in intergroup interaction studies. In most Western contexts, overtly discriminating against someone because of their race/ethnicity carries social and legal costs. However, it is unlikely that racism, xenophobia, and fear of "different or exotic others" simply vanished when cultural sanctions against these biases were set in place. Instead it is likely that for some people the lingering effects of racism remain, but are masked by conscious actions and explicit statements. What remains are the uncontrollable responses that may be manifested via physiological signals.

There is no question that self-reported shifts in attitudes towards minorities have become increasingly egalitarian over the past half century – in 1958, for example, 38% of voters reported that they would vote for a qualified African American for president; by 2003 the number was 92% (Gallup, 2004, see Keeter & Samaranayake, 2007). But self-reported attitudes represent one facet of an individual's belief structure the component they are willing to share with others. Because they cannot be explicitly controlled, except in rare circumstances, physiological responses may reveal reactions to target others or taboo stimuli that indicate a different mental state than the one that individuals are willing to self-report. For example, Blascovich, Mendes, and Seery (2002) report that while participants' explicit ratings of experimentally facially birthmarked others were significantly more positive on variables such as attractiveness, intelligence, etc. than the same targets without birthmarks, they exhibited a threat response physiologically to the former but not the latter.

Changes in physiological responses indexing a specific mental state may precede conscious awareness of that state and thus may be *prognostic*. Such changes may be detectable prior to explicit reportable mental states. Strong evidence for this idea comes from Demasio and Bechara's study examining decision-making using a gain/loss card game (Bechara, Damasio, Tranel, & Damasio, 1997). In this study, skin conductance level was measured while participants made choices of which of four decks of face-down playing cards from which to turn over a card. Cards turned over from two of the decks produced larger overall losses than gains when the participant's goal was to optimize gains. When asked explicitly which decks were the better decks, normal (non-brain damaged) participants guessed correctly by approximately the 40th trial. However, skin conductance levels increased prior to participants choosing from loss decks by approximately the 10th trial, suggesting that bodily responses indicating loss preceded conscious awareness of that loss.

Finally, physiological responses might provide information about *how* mental states influence behavior (or vice versa). That is, physiological responses might be the causal explanation and be thought of as *mechanistic*. This argument can be seen in a variety of research programs. In medical research and health psychology studies of stress, ANS and/or endocrine responses are often regarded as the mediator via which individual or group differences are linked to end-point physical health outcomes, like essential hypertension or coronary artery disease (see Blascovich & Katkin, 1993). For example, researchers examining the influence of personality factors on cardiac diseases have suggested that individuals higher in dispositional hostility have exaggerated increases in blood pressure responses (Chesney & Rosenman, 1985) or cardiac activity (Matthews, 1988) during mildly stressful tasks, and this acute reactivity is implicated as part of the etiology of vascular and cardiac diseases.

To be sure, statistical mediation does not necessarily identify the casual *mechanism* for how individual differences lead to physical health outcomes. For example, blood pressure responses, like most ANS responses, are imperfect measures and are often end-points of complex physiological processes that are difficult to measure directly – for example, blood pressure is determined by overall vascular resistance and cardiac outflow. So, although pointing to physiological responses as statistical mediators between a personality trait on one hand and end-points of health or behavior on the other hand, is a potentially fruitful avenue of study, one needs to be careful to not over-state mechanism claims.

Physiological Indexes of Psychological States

Scholars have intuited relationships between bodily states and mental constructs for millennia. Nevertheless, scholars in Western cultures eschewed physiological measures well into the twentieth century due in large part to the lingering metaphysical

assumption of the Cartesian notion of mind–body substance dualism. However, beginning in the middle of and lasting to the end of the twentieth century, social psychological researchers began taking seriously the notion of mind–body relationships.

These early attempts remained generally in the form "A difference in physiological response X occurs when independent psychological variable Y is manipulated." For example, on the basis of an observation that a person's heart rate increased when he or she viewed photographs of another person as opposed to a non-sentient object might have led an investigator to conclude that such heart rate increases measured passion or even love (e.g., Valins, 1967). The physiological rationale underlying such conclusions was that the human psyche is somehow tied closely to the autonomic nervous system, particularly to its sympathetic branch that was thought to be activated during periods of some sort of psychic arousal or disequilibrium. Hence, many autonomic measures, particularly sympathetic ones, were regarded as redundant and interchangeable.

The validity problems with this approach were at least twofold: it relied on a relatively naive physiological assumption; and, researchers fell into the logical "reverse inference" or "affirmation of the consequent" trap. The former was somewhat excusable as the assumptions of parallel sympathetic anabolic processes (i.e., activation of one process indicated activation of all) and catabolic tension between sympathetic and parasympathetic processes (i.e., the latter tending to diminish the former) were regarded more as fact than hypothetical. However, "affirmation of the consequent" is inexcusable as the presence of a supposed physiological response (i.e., consequence) does not guarantee the presence of a psychological phenomenon except in very limited and special circumstances.

The nature of relationships between any measures or indexes, whether physiological, behavioral, or subjective in nature, of psychological states and processes and the states and processes themselves always bear scrutiny. Problems of reverse inference or affirmation of the consequent are not distinctive to physiological indexes as we know; for example, from methodological concerns about social desirability problems with paper and pencil measures. However, it is important to remember that there is no validational shortcut just because indexes may be physiological.

Cacioppo and colleagues have clarified (Cacioppo & Tassinary, 1990) and re-clarified (Cacioppo, Tassinary & Berntson, 2007) the taxonomy of relationships between psychological and physiological variables assuming the identity thesis; that is, that all psychological processes are somehow embodied. Blascovich (2000) summarized their arguments as "... the more one limits the social psychological construct, and expands the set of physiological measures indexing it, the closer the construct and index can come to an invariant relationship;" that is, one cannot occur without the other.

Cacioppo et al. (2007) specified that an investigator can determine the strength of the relationships between psychological variables and physiological responses

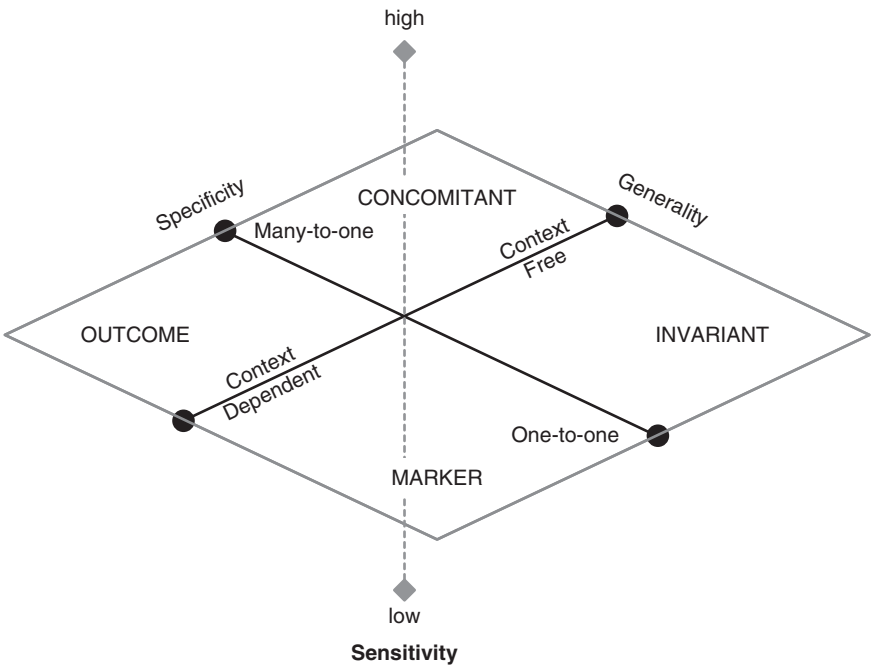


Figure 1.1 Cacioppo et al. Taxonomy of Psychophysiological Inference

by considering three dimensions or axes. These include *generality*, *specificity*, and *sensitivity*. Plotting these as orthogonal to each other produces a three-dimensional model with four segments varying in sensitivity (see Figure 1.1).

Generality refers to a contextual continuum on which indexes vary from limited or "context-dependent" to unlimited or "context-free." The latter are more desirable because the physiological indexes are generalizable across all situations (i.e., contexts). However, the latter are also relatively and even extremely difficult to achieve. Nonetheless, even context-dependent indexes can be valuable if the theoretical domain is similarly limited though still important or valuable.

Specificity involves the relationship between the index and construct. Perfect specificity means that there is a one-to-one relationship between a construct and its index. When the construct is activated, the index is activated and vice versa. When the construct is inactive, the index is not active and vice versa. In Cacioppo et al.'s (2007) nomenclature, specificity varies from "one-to-one" to "many-to-one." The latter means that any of several constructs can lead to the same index, and therefore cannot be distinguished form each other via that index. It is important to note, however, that "one-to-one" does not necessarily imply that a single physiological measure such as heart rate, cortisol level, or specific muscle response is necessary for an index to have a one-to-one relationship with a

psychological construct. Importantly, a "single" physiological index can be defined as a pattern of multiple physiological measures at a point in time or even a pattern of physiological measures over time (i.e., topological pattern).

Sensitivity in the typology of Cacioppo et al. (2007) is the degree to which a physiological index corresponds to the putative underlying psychological state or process. Sensitivity provides information about the degree to which changes in the physiological index reflect the changes in the underlying psychological state or process.

Using the 2 2 2 typology based on generality, specificity and sensitivity, four segments are possible. That measures falling into the various segments vary in their logical strength as indexes does not mean that any segment is valueless. However, investigators must be aware of the limitations in terms of generality, specificity, and sensitivity of their own created or adopted physiological measures or indexes of psychological constructs.

For example, the validity claims of Blascovich and colleagues (e.g., Blascovich & Tomaka, 1996; Blascovich & Mendes, 2000; Blascovich, 2008) regarding their multivariable-based physiological indexes of challenge and threat motivation are limited to active coping or "motivated performance situations;" that is, ones requiring task engagement and instrumental cognitive responses such as exams, speeches, games, interviews, etc. and not necessarily to passive coping situations such as watching scary movies, witnessing crimes, etc. Yet, these markers have proven highly sensitive to motivational levels across a variety of social psychological processes (e.g., Blascovich, 2008), and they are important to the extent that motivated performance situations pervade human and social lives.

Inferring Psychological Constructs from Physiological Responses

The logic of developing and inferring properties is important, but it is also important to consider what can be done to maximize those properties. Blascovich and Seery (2007) proposed a set of principles for increasing the strength of inferences made from physiological measures or indexes of social psychological constructs.

Principle 1: Specify the nature of the construct

Because social psychological constructs are often labeled with common language terms (e.g., attitude, self-esteem, risk taking, ego depletion, threat, stress, liking, prejudice) sometimes researchers assume that everyone agrees on what a construct means. Indeed, it can be argued that the reverse is true. Because social psychological constructs are often labeled in common language terms, their meaning is often left implicit or fuzzy or both. Science demands precision not

only in terms of methods and measurement but also in terms of definitions of terms and constructs (Blascovich & Ginsburg, 1978). Common language connotations or even "dictionary definitions" of constructs are not enough. One must be as specific as possible regarding what one means by scientific constructs. This does not imply universal agreement, which though desirable is not absolutely necessary, only that the investigator is clear to other researchers and consumers about what he or she means by the construct.

Principle 2: Specification of physiological indexes

At the present time, the control of physiological processes has been assumed to be largely a central nervous system process (i.e., the brain and its projections to the cranial endocrine organ – the pituitary). It is also the case that peripheral embodiments themselves (Blascovich & Mendes, 2010) have a role to play on moderating and perhaps even mediating such control through various hormonal and afferent neural influences. These systems clearly interact cybernetically.

Nevertheless, we are concerned here with peripheral physiological and endocrine processes as candidate indexes for social psychological constructs. Such indexes have several advantages. First, arguably most if not all of the results of central nervous system and endocrine processing are expressed peripherally, which are orders of magnitude less complex than their central nervous system antecedents. Second, the costs of peripheral measures for investigators is currently much less than the costs of central measures, especially ones based on brain scanning. Third, they are less intrusive. Fourth, participant embodiments (i.e., movements) are less restricted. Fifth, the predicted peripheral physiological indexes of social psychological constructs can be measured more precisely than putative underlying central nervous system and endocrine ones.

Principle 3: Specification of theoretical physiological linkages

Because neither the first nor second principles above are sufficient to postulate causal linkages between social psychological processes or constructs and candidate peripheral physiological linkages, researchers must specify theoretical mechanisms and processes for linking the two. In many ways, the power of the physiological index rests on the validity of the underlying theoretical rationale. Because of the importance of establishing one-to-one relationships between physiological indexes and psychological constructs, and because very few if any peripheral physiological responses are not mediated or moderated by a cascade of other physiological factors, it is very likely that worthwhile indexes can only increase in specificity and sensitivity if they include multiple responses and, often, are measured over time.

Going Forward with this Book

The critical take-away points from this discussion are that researchers should approach measurement and interpretation of neurophysiological indicators carefully, soberly, and somewhat skeptically. Autonomic responses neither provide a royal road to the truth nor are self-report or behavioral data lacking in value. Ideally, all three measurement categories should be part of a multi-method approach. Also, there are occasions when physiological responses are expected to correlate with self-report responses and occasions when they are expected not to correlate. The advantages described above describe a possible road map to begin to make speculations about relationships across physiological and non-physiological methods.

The rest of this book focuses on three types or categories of physiological response measures worthy of researchers' interest. Each category comprises a chapter that was designed to stand on its own. The fact that each of the three chapters corresponds closely to the work of at least one of the authors of this book is no accident. Indeed, it would be impossible to single-author a work such as this because specialized and practical knowledge in each area is so important. Yet, it is also important to realize the commonalities across various categories of physiological measures.

A final caveat is that this is not a "cook book." Rather, it is a distillation of knowledge and experience that the authors have found has promoted and defined their own use of social psychophysiological approaches. The book was written to educate readers in both a general and a specific way. However, there are many other sources available to help researchers include endocrine and peripheral neurophysiological measures in their research. Not the least of these are the many guidelines for specific physiological measures published by the Society for Psychophysiological Research in its journal *Psychophysiology*.

2

Autonomic nervous system: Obtaining, quantifying, and interpreting peripheral physiological responses

In this chapter we explore the primary regulatory system of the body – the autonomic nervous system (ANS) – which functions to mobilize energy and deliver oxygenated-blood to the body. Changes in the ANS can result from such quotidian events as sleeping and waking, postural changes, and physical movement. More interestingly for social psychologists, ANS responses can change as a function of shifts in mental states involving emotional, attentional, motivational, and stress-related processes. Here, we review some of the more commonly used measures that can be obtained relatively non-invasively. We describe the process of obtaining ANS responses including technological requirements for lab and field experiments, and the specifics of obtaining, scoring, quantifying, analyzing, and interpreting ANS responses. We end each subsection by reviewing social psychological literature that has effectively used these measures.

Getting Started

Like riding a bike for the first time, getting started with a psychophysiology lab might be the most difficult part. Many decisions must be made regarding how to set up a laboratory, what equipment to purchase, what type of space is needed, programming versus purchasing software to edit and score data, and more. This section describes some of the choices that need to be made and provides information to assist in making these choices. Also, it is important to point out that a "best-case scenario" is presented and there will be occasions when one will not be able to control all the elements in a dedicated space. In this case, it is important to know what could affect recordings and to faithfully keep track of those factors as possible extraneous influences.

The question of what type of equipment one should obtain needs to be guided by what types of research questions will be explored. Researchers should not find themselves in the position of developing research questions around the equipment

they have, but rather, the research questions should dictate the physiological equipment they obtain. Here, general guidelines for setting up a lab are described, delaying discussion of specific measures until the following section.

In order to set up a social psychophysiology lab one should first consider a couple of issues. Generally, as for all human behavioral science laboratories, noise and comfort are important. Laboratories should be climate controlled, allowing a comfortable range of ambient temperatures. They also should be free of distractions in the form of sights and sounds from both lab equipment and personnel and the larger external environments (e.g., buildings and surroundings) in which they exist. For experiments in which participants are sitting, be sure that the participant's chair is comfortable and has arms on which participants can rest hands and arms comfortably especially if there are sensors applied to them. Some blood pressure monitors, for example, require the sensor to be placed on the wrist (e.g., tonometric technology) and even slight changes in arm elevation can influence the readings.

Regarding the lab space, a private room to apply sensors to participants is ideal. In some cases, the application of sensors requires participants to partially disrobe – exposing torsos, unbuckling pants – that can best be accomplished with privacy. This room can double as the testing room in which the participant remains for the study or a separate one, but a room in which two people – the experimenter and the participant – can move about without interference from equipment, furniture and each other. Separate or not, the *testing* room, where the experiment takes place, should be relatively comfortable and quiet because for most studies one needs to obtain quiet resting baseline data prior to beginning the formal experiment.

For most experiments, baseline data are typically obtained while participants are seated. However, if for some reason, participants will be standing or supine during the formal experiment, one would want these positions replicated during the baseline period. If an experiment necessitates a reclining position – for example, a peripheral physiological study to complement a neuroimaging study – then obtaining a reclining baseline to compare responses is necessary. This is because body posture can not only influence blood flow through the heart, but might also influence emotional and mental states (Harmon-Jones & Petersen, 2009; Mendes & Barrett, 2010). For example, Harmon-Jones & Petersen recently reported that participants who were reclined showed fewer shifts in EEG left frontal asymmetry during an anger evocation compared to those sitting upright.

In the next section, we turn to the topic of equipment and various ANS measures that can be collected. We describe various ways to collect, edit, and quantify these responses. For organizational purposes the various measures are separated into cardiac responses (electrocardiography and impedance cardiography), hemodynamic responses (blood pressure), and peripheral responses (skin conductance). At the end of each of these sections we review social psychological research that has capitalized on these measures. For a summary of the measures, definitions, and common modes of collection see Table 2.1.

Table 2.1 Descriptions of commonly used ANS measures and sources of the responses

Common abbreviations of ANS measures and their unit of measurement	*Definition*	*Equipment to source the measure*
HR (bpm)	Heart rate typically reported in beats per minute.	Pulse meter or Electrocardiograph
IBI (ms)	Interbeat interval reported in milliseconds. IBI is the preferred measure of chronotropic cardiac activity	Electrocardiograph
SV (ml)	Stroke volume measured in milliliters. It represents that amount of blood ejected on each heart beat.	Electrocardiograph and Impedance cardiograph *or* Phonocardiogram
CO (L)	Cardiac output measured in liters. Calculated as: HR × SV = CO Represents the amount of blood ejected from the heart during one minute	Electrocardiograph and Impedance cardiograph *or* Phonocardiogram
PEP (ms) (aka VC)	Pre-ejection period (also referred to as ventricle contractility) is a time-based measure that is determined as the time from the left ventricle contracting to the opening of the aortic valve. These two time points are often referred to as from point Q (on an ECG trace) to point B (on a dz/dt waveform) Ventricle contractility is a different score that is calculated from two different PEP measures and multiplied by −1 so that increases in VC represent increases in SNS. $PEP_{task} - PEP_{baseline} = PEP_{change}$ $PEP_{change} \times -1 = VC$	Electrocardiograph and Impedance cardiograph *or* Phonocardiogram
LVET (ms)	Left ventricular ejection time is a time based measure determined from the B point on the dz/dt wave to the x point on the dz/dt wave	Impedance cardiograph
HI (ohm/sec2)	Heather Index is a measure of aortic contractility. It is derived as the ratio of dZ/dt max to Q-Z interval (electromechanical time interval). This index has been shown to be especially sensitive to changes in cardiac contractility	Electrocardiograph and Impedance cardiograph
EMS (ms)	Electrical mechanical systole is the total time from the left ventricle contracting to the aortic valve closing. Determined from the Q point on the ECG to the X point on the dz/dt or by adding PEP + LVET.	Electrocardiograph
T wave amplitude (volt)	T-wave amplitude is the change in amplitude of the T-wave between tasks. Increases in T wave are thought to be related to more SNS activity.	Electrocardiograph

Table 2.1 (Continued)

Common abbreviations of ANS measures and their unit of measurement	*Definition*	*Equipment to source the measure*
SBP (mmHg)	Systolic blood pressure measured in millimeters of mercury. SBP refers to the maximal blood pressure and occurs when the ventricles of the heart contract.	Blood pressure monitor
DBP (mmHg)	Diastolic blood pressure measured in millimeters of mercury. DBP refers to the minimal blood pressure and occurs when the ventricles are most relaxed.	Blood pressure monitor
PP (mmHg)	Pulse pressure measured in millimeters of mercury. Represents the difference between maximum blood pressure and minimum blood pressure. Calculated as: SBP-DBP = PP	Blood pressure monitor
MAP (mmHg)	Mean arterial pressure in millimeters of mercury. MAP refers to a type of average of blood pressure, but in this case SBP and DBP are not weighted equally, DBP is weighted more. One formula used to calculate MAP is: 1/3(SBP – DBP) + DBP = MAP	Blood pressure monitor
TPR (resistance units)	Total peripheral resistance is a measure of the overall resistance in the vasculature, specifically the arterioles. Along with cardiac output, TPR is the determinant of BP. Because TPR is difficult to measure directly it is derived using the following formula: (CO/MAP) × C = TPR C=constant, typically 80	Electrocardiograph and Impedance cardiograph and Blood pressure
RSA (ms)	Respiratory sinus arrhythmia (aka high frequency heart rate variability, HF HRV) is a type of heart rate variability in which spectral analysis is used to derive the high frequency component of the IBI cycle (.12 to .40 Hz).	Electrocardiograph
SDNN	Standard deviation of normal to normal heart beats. A measure of heart rate variability defined as the standard deviation of Interbeat intervals.	Electrocardiograph[a] Blood pressure Photoplethysomograph
RMSSD	Root mean square of successive differences. A measure of heart rate variability calculated as the square root of the mean squared difference of successive normal to normal heart beats.	Electrocardiograph[a] Blood pressure Photoplethysomograph
RR	Respiration rate refers to the number of breaths per minute that occur in the high frequency range (typically 12 to 20 breaths per minute).	Respiration band *or* Impedance cardiograph

(Continued)

Table 2.1 (Continued)

Common abbreviations of ANS measures and their unit of measurement	*Definition*	*Equipment to source the measure*
RA or RD	Respiration amplitude or depth refers to the difference in chest circumference during inhalation compared to exhalation.	Respiration band *or* Impedance cardiograph
SCR (μS)	Skin conductance reported in microSiemens. Indicates the amount of activity in the eccrine glands and is tied to a specific event or stimulus.	Skin conductance
NS-SCR	Non-specific skin conductance responses are reported in terms of the number of these events per minute (typically between 1 and 3)	Skin conductance
SCL(μS)	Skin conductance reported in microSiemens. Indicates the amount of activity in the eccrine glands and, unlike SCR, is a time based measure of overall level of SC.	Skin conductance
FPT(ms)	Finger pulse transit time reported in milliseconds is determined by the time between the left ventricle contracting (Q-wave on ECG) and the height of a pulse wave form at the finger. Shorter FPT indicates that the pulse detected at the finger relative to the heart contracting traveled faster than longer FPT. FPT is inversely (though not perfectly) related to blood pressure.	Electrocardiograph *and* Photoplethysmograph attached at finger
FA	Finger pulse amplitude refers to height or amplitude of the pulse waveform detected at the finger. Typically examined as changes (reactivity) from one task (e.g., baseline) to another. Increases in FA indicate more local dilation of the vessels in the finger, whereas decreases in FA indicate local constriction.	Photoplethysmograph attached at finger
EPT	Ear pulse transit time, similar to FPT, is a time based measure determined from the left ventricle contracting (Q wave on ECG) to the height of the pulse wave at the ear	Electrocardiograph *and* Photoplethysmograph attached at finger

Note. [a] The preferred source for the measure

Cardiovascular (CV) Measures

In simplest terms, the cardiovascular (CV) system consists of the heart and pathways (vessels) through which oxygenated blood is delivered to the periphery and

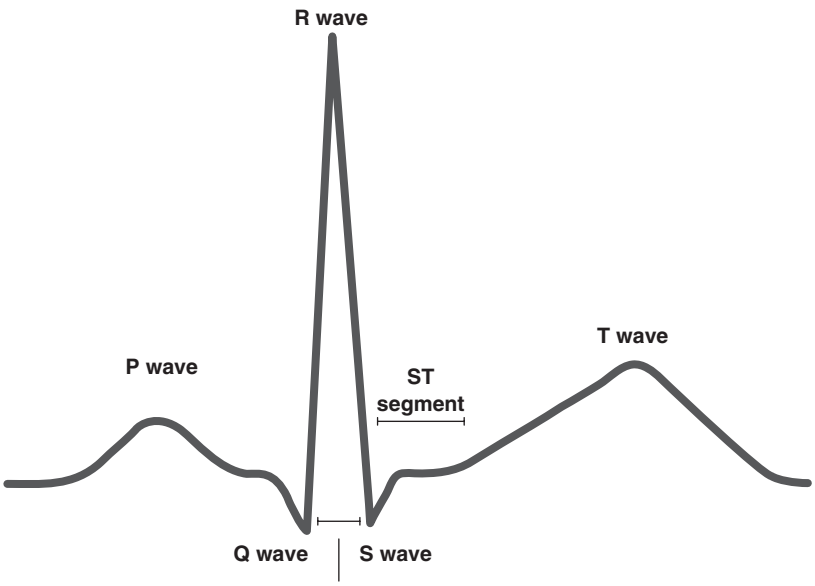


Figure 2.1 ECG waveform

deoxygenated blood returns to the heart. Importantly to social psychologists, this system is responsive to affective states, motivation, attention, and reflexes. Additionally, CV responses have been linked to vulnerabilities in physical and mental illness. In this section we review several methods that examine changes in the cardiac cycle: electrocardiogram, respiration, and impedance cardiography.

Electrocardiograph and respiration

The heart produces an electrical signal that can be measured via an electrocardiogram (ECG). A normal ECG recording is composed of various inflections (i.e., changes in direction or slope) referred to as P, Q, R, S, and T waves (Figure 2.1). Each heart cycle begins with an electrical impulse from the sinoatrial node (not detected on the ECG wave), which results in a depolarization of the atria (P-wave). The QRS complex represents the depolarization of the ventricles and the T inflection indicates repolarization (or recovery) of the ventricles. These inflections in combination can be used to determine a variety of chronotropic (i.e., time-based) measures such as the time of one complete heart cycle, known as heart period (or interbeat interval [IBI]). This measure is the inverse of heart rate (beats per minute), though heart period is the preferred metric because of its statistical properties (see Berntson, Cacioppo, & Quigley, 1993).

Equipment

There are many equipment options available for ECG recording and it is preferable to record the ECG waveform rather than obtaining a summarized data point like heart rate (HR). Though not completely uninformative, simply collecting HR and mean inter-beat interval (time between the R-points) limits the researcher's ability to calculate more sophisticated measures such as heart rate variability (see below, and Table 2.1). In other words, it is relatively easy to collect the full ECG waveform and the benefits outweigh the costs.

Preparation and recording

Electrocardiogram waveforms can be collected from several spot lead configurations of sensors (e.g., 35 mm electrodes) on the limbs. Described below are three standard configurations in which placement results in an upward deflection of the Q-R complex:

Lead I: Electrodes are attached just above the right and left wrists on the inside of the arms. The left arm has the positively charged lead.
Lead II: Electrodes are attached on the right arm and left ankle. The ankle has the positively charged lead.
Lead III: Electrodes are attached on the left wrist and left ankle. The ankle has the positively charged lead.

Lead placements can be adjusted so that the sensors are placed on the torso rather than the limbs. For example, a modified Lead II configuration places the right lead below the sternum and the left lead on the left side of the torso below the ribcage. Torso placement might be preferable over limb placement if there is anticipated movement of the limbs or for younger participants (i.e., babies and toddlers). In Figure 2.1, the participant has an ECG placement in a standard lead II configuration – right arm, left leg, right leg ground. He is also wearing a respiration band to track rate and depth of this breathing. This configuration is appropriate for obtaining heart rate variability (see more details below). Note that in this case we did not shave his legs to apply sensors. Though it would be preferable to have hairless skin, the ECG wave is especially strong and can be reliably obtained even with interference from hair.

The experimenter should apply sensors to participants. This is done to insure proper and consistent placement across participants. To ease any participant discomfort, a same-sex experimenter is typically employed. Preparing the site for ECG placement can include a gentle abrasion of the skin and a subsequent application of a thin layer of conductance gel, but in many cases a clean signal can be obtained without either, given the relatively strong electrical signal of the heart.

Several factors can interfere with an ECG recording that should be anticipated. First, excessive hair, either on the ankles or chest, can make recording difficult if using adhesive sensors. Shaving participants' ankles or torso might be possible, but could be problematic in some situations. Either adjusting the sensor location or using additional medical tape to secure the sensor might reduce noise. Another potential problem is participant's skin type or changes in skin temperature during the course of the experiment. Skin that is especially oily or prone to sweat might require additional taping of disposable sensors. Good lab practice includes taping the sensors with medical tape, and this is especially true in summer months or for longer studies, when the risk of warmer skin temperature is greater.

Collecting the signal: sampling speed, filters and amplification

Typically equipment (hardware) for collecting ANS responses allows for a specification of sampling rate. This is the number of samples per second that the computer records. Choosing sampling rate should be determined by the measure being collected – with slow-moving waves one can have a slower sampling rate, but with faster moving signals one needs a faster sampling rate. For example, skin temperature is a slow moving signal – changes in skin temperature are relatively slow – and thus can be sampled at a lower sampling rate like 200 Hz or 200 samples per second. In contrast a quick-moving signal like ECG should be sampled at a higher sampling speed – 1000 Hz – so that all the possible inflections and deflections are properly traced.

Typical equipment also allows for a selection of filters. The function of filters is to remove or reduce portions of the signal that are irrelevant for the signal of interest. There are four common types of filter: low pass, high pass, bandpass, and notch filters. A low pass filter allows frequencies *below* a set frequency value to pass, whereas a high pass filter does the opposite – frequencies *above* a set frequency are allowed to pass. For example, the alpha rhythm from EEG ranges from 8–12 Hz. To insure that only signals in this frequency range are collected an experimenter could set a low pass filter to 12 and a high pass filter to 8, leaving only the desired frequency range for collection. A bandpass filter is simply a filter with low and high pass settings. A notch filter is a filter that attenuates a small range of frequencies. The most common notch filters are ones that attenuate AC current. In the US, AC current is set at 60 Hz, so a 60 Hz notch filter is often used. For ECG the typical frequency is 1 Hz (one cycle per second, 60 heart cycles in one minute) so a bandpass filter set at 0.5 Hz and 50 Hz would insure that very low and very high heart rates would be properly obtained.

Typically, while the ECG trace is being collected (online) or afterwards (offline), the ECG waveform needs to be appropriately amplified. Amplification is the process of adjusting the strength of the signal so that a specific intensity of the signal is obtained. For most physiological signals discussed in this chapter

the optimal amplification for the signal is 1 volt. Because the intensity of any waveform may change during the course of an experiment, one should amplify the signal at the beginning of the experiment, prior to baseline. This is accomplished by adjusting the *gain* on the amplifiers. If post-acquisition software will be used that allows adjusting the amplification post-acquisition (e.g., Acknowledge software, produced by Biopac, allows this option), collect a small amount of data prior to the beginning of baseline or use the first minute of the data collection (presumably a baseline period) to determine how much the signal needs to be amplified.

Editing and quantification: ECG and HRV

Editing an ECG waveform is typically done offline – that is, once the session is complete. The primary concerns when editing an ECG waveform are the removal of artifacts and the proper identification of the R inflection. Another critical point on the ECG waveform is the Q inflection – or the point at which the left ventricle of the heart contracts. The Q inflection, along with the B inflection from the $\Delta z/\Delta t$ impedance wave (see impedance cardiography section), is critical for the calculation of pre-ejection period (PEP), which is one of the purest measures of sympathetic activation of the heart.

Collection of the ECG trace also allows for the estimate of heart rate variability. HRV is influenced by a number of factors, but by deconstructing the variability one can isolate heart period changes due primarily to parasympathetic control, sympathetic control, or a combination of both. Of particular interest to psychophysiologists is high-frequency (HF) HRV because changes in variability in this range are believed to be due primarily to control of the vagus nerve and thus primarily an index of parasympathetic control. There are several measures of HRV estimates, time-domain, frequency domain, and non-linear measures (a full committee report by the Society for Psychophysiological Research is available for more details: Berntson, Bigger, & Eckberg, 1997). Here we briefly review some of the estimates and what is needed to calculate these measures.

One of the simpler measures of HRV is based on time-domain estimates, for example RMSSD (root mean square of successive R-R differences), which is calculated as the standard deviation of the beat-to-beat intervals. A popular frequency-domain technique to estimate HRV involves decomposing heart period variance into different frequency bands using Fourier transformations. For example, the HF band (high frequency band) ranges from 0.15 to 0.4 Hz (cycles per second) and is thought to represent primarily vagal influence, and as such parasympathetic activity. Lower frequency bands (< 0.15) have also been identified, and in these frequency domains the influence can be either sympathetic or parasympathetic.

Respiration can influence heart rate and heart rate variability. In Figure 2.2, the participant is wearing a respiration band on his chest to monitor both his respiration

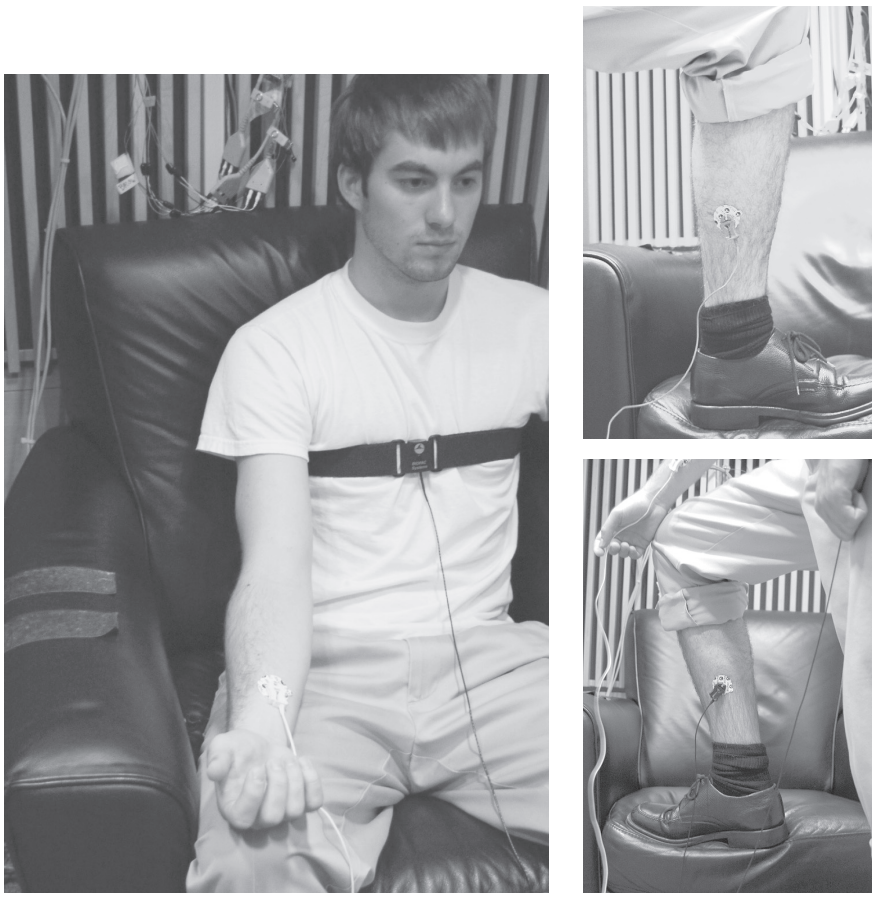


Figure 2.2 Standard lean II configuration for electrocardiograph (ECG) with respiration band (Photos: Christopher Ovies)

rate and the depth of his breath. Collecting respiration parameters are especially important if one wants to measure indicators of heart rate variability (HRV) because respiration can directly influence HRV – during inspiration the influence of the vagus nerve on the heart is removed and the heart rate accelerates; during expiration, the vagus nerve is applied and heart rate decelerates. One commonly debated measurement issue in HRV research concerns the importance of controlling for respiration rate and depth in HRV analysis. For a thorough understanding of the complexities of this issue, see Denver, Reed, and Porges (2007) for justification that respiration frequency need not be included in estimates of RSA/HRV, and Grossman and Taylor (2007) for a discussion of why respiration frequencies are important.

Respiration can be measured a number of ways. One option is to use a strain gauge that measures pressure during inspiration and expiration, and rate and depth of breaths can be extracted. With a single strain gauge the recommended placement is high on the torso immediately under the arms (and above the breasts). This placement will allow for measurement of upper respiration, but not lower abdominal respiration, which may be important if the research focuses on deep breathing found in meditation or other focused breathing domains. In this case, two strain gauges can be used to provide both upper and lower respiration. Another option is to use impedance cardiography, which can extract respiration rate and depth.

Applications of heart rate variability in social psychology

Initially, heart rate variability was believed to be a measurement artifact or nuisance, but further exploration into spontaneous changes in the timing of the heart cycle proved to be psychologically and physiologically meaningful. Though there are still disagreements on the specifics related to measurement, quantification, and psychological meaningfulness of vagal tone and cardiac vagal reactivity (see *Biological Psychology*, 2007, vol 74), these measures might prove to be especially important for social psychologists interested in emotion and/or mental effort.

Though most work has focused on resting/baseline RSA (a type of HF heart rate variability) and its links to dispositions and responses to social and emotional situations, there is also a growing literature on vagal reactivity – focusing on RSA changes – and vagal rebound. Vagal rebound is the extent to which RSA responses return to or even over-shoot baseline levels after some suppression of the vagal brake. Below we describe some literature exploring these various components of HRV.

One theory that has received much attention in terms of the inferences one can draw from heart rate variability is Porges' polyvagal theory (e.g., Porges, 2007). In this theory, Porges argues that vagal regulation stemming from the nucleus ambiguous and enervation from cranial nerve X acts on the vagus nerve to modulate heart period. The polyvagal theory further specifies that primates uniquely have vagal nerve modulation (but see Grossman & Taylor, 2007), which has evolved as part of the social engagement system. One of the primary postulates of polyvagal theory is that social factors (affiliation, social engagement) or personality factors (optimism, bonding, compassion) can modulate vagal activity. Specifically, Porges argues that higher RSA (high cardiac vagal tone) can be used as an index of adaptive emotional regulation and responsiveness to the social environment. Similarly, cardiac vagal reactivity might also index appropriate social engagement in that increased vagal reactivity might be associated with calmness, equanimity, and a lack of distress.

Adding some complexity to these effects, however, is the nature of the social context. Indeed, in highly stressful situations or tasks that require mental attention

or effort, one should expect a withdrawal of the vagal brake (resulting in lower RSA). In fact, cognitive psychophysiologists have used decreases in RSA as an index of attention or mental effort (Tattersall & Hockey, 1995). In one study, relying on this type of interpretation for HRV reactivity, Croizet, Després, and Gauzins (2004) examined changes in RSA during a stereotype threat paradigm. They found that participants assigned to a stereotype threat prime had greater decreases in RSA and poorer performance than those in the control condition and that RSA changes mediated the relationship between stereotype threat and performance.

Applications of cardiac vagal tone and vagal reactivity are increasing in personality and social psychology. Some applications have focused on the extent to which dispositional emotional styles are linked with cardiac vagal tone (Demaree & Everhart, 2004; Oveis, Cohen, Gruber, Shiota, Haidt, & Keltner, 2009; Sloan, Bagiella, & Shapiro, 2001). For example, individuals with greater hostile tendencies have lower cardiac vagal tone at baseline, during an emotional induction task, and at recovery than those lower in hostile tendencies (Demaree & Everhart, 2004; Sloan et al., 2001). Similarly, but on the brighter side, Oveis and colleagues found that those higher in optimism had higher vagal tone. Accumulating evidence suggests that vagal tone might be a reasonable physiological response to index general positive and negative affect.

Changes in RSA (RSA reactivity) have been implicated as a possible mediator for why implicit goal setting might result in improved performance. In previous studies, participants who exaggerated reports of their GPA tended to improve more than those who did not exaggerate (Willard & Gramzow, 2008). However, an open question was whether exaggeration was benign and serving a type of implicit goal setting or was exaggeration a form of anxious repression. To examine this question, participants first reported their GPA and course grades in private and then met with an experimenter to review their academic history (Gramzow, Willard, & Mendes, 2008). During this interview participant's ECG and respiration was recorded and RSA responses were calculated. Participants who exaggerated their GPA showed greater RSA increases from baseline to the interview, suggesting that participants who exaggerated their GPA were not necessarily anxious about exaggerating their achievements. Additionally, those who had greater increases in RSA when discussing their GPA tended to improve their GPA in a subsequent semester. Converging evidence from nonverbal behavior coded during the interview suggested that exaggerators appeared composed rather than anxious, supporting the interpretation that higher RSA while discussing one's GPA was associated with equanimity rather than anxiety.

Impedance cardiography

Impedance cardiography is a non-invasive technique to estimate blood flow changes in the heart. This technique allows for estimates of how much

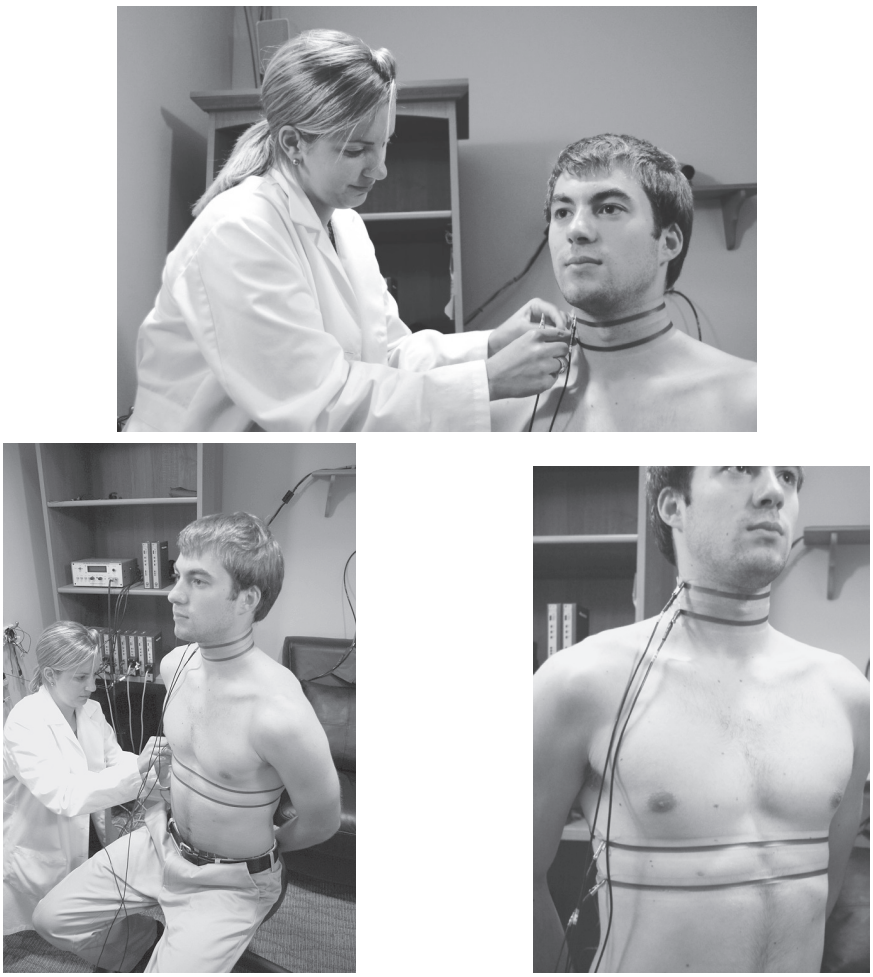


Figure 2.3 Band electrode placement for impedance cardiography (Photos: Christopher Ovies)

blood is ejected during each heart cycle (stroke volume; SV), and various changes in the cardiac cycle such as the timing of the aortic valves opening and closing.

Impedance cardiography requires the use of either spot or band electrodes[1] placed on the torso. In Figure 2.3, the participant is wearing band electrodes that completely encircle his neck and torso. Two bands are placed around his neck and two around his torso, with the upper torso band placed directly on his xiphisternal junction (i.e., right under the sternum), and the lower torso band placed 3 cm below the upper band. Similarly, the lower neck band is

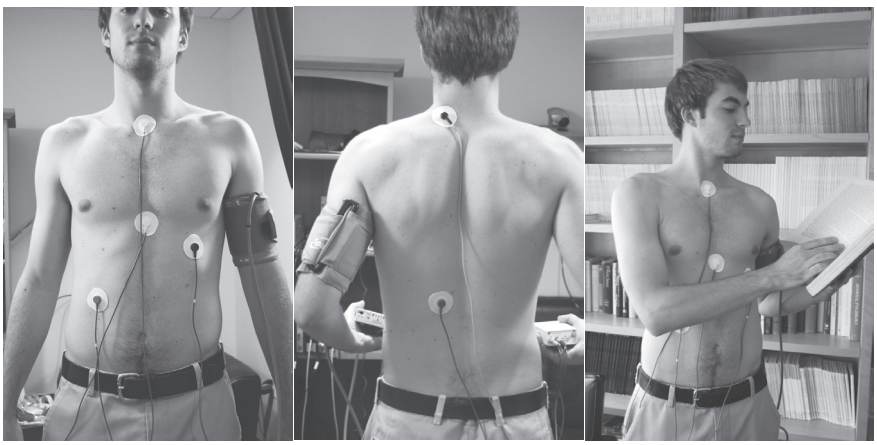


Figure 2.4 Spot electrode placement for impedance cardiography and electrocardiography (Photo: Christopher Ovies)

placed low on the neck and the upper neck band is placed 3 cm above that. Impedance cardiography employs an output of frequency modulated electrical current (ranging from 0.1 to 4 mAmps) to the two outer sensors (upper neck and lower torso), and the inner sensors detect the impedance (i.e., AC resistance) to the incoming current. Impedance values represent global blood flow in the thoracic cavity (typically referred to as Z_0 or basal impedance). As the blood volume increases, the impedance decreases. The first derivative of the waveform, $\Delta z/\Delta t$ (Δ indicates delta or change, so $\Delta z/\Delta t$ is defined as a change in basal impedance [z] over a change in time [t]), is the change in basal impedance over the change in time, which provides a waveform that allows for an estimate of the total amount of blood volume ejected from the heart on a single beat (i.e., stroke volume).

An impedance spot electrode placement is shown in Figure 2.4 using an ambulatory impedance machine (VU-AMS). This 6-spot sensor configuration allows for collection of both ECG waveform and $\Delta z/\Delta t$ waveform. The specific placement of these sensors is:

1 ECG: over the jugular notch of the sternum, between the collar bones.
2 ECG: under the left breast, 4 cm under the nipple, between two ribs rather than on a rib.
3 ECG: at the right lateral side, between the two lowest ribs.
4 ICG: over the xiphoid process of the sternum.
5 ICG: at the base of the neck (vertebrae C3/C4) and at least 3 cm above electrode 1.
6 ICG: below the line connecting the tips of the shoulder blades (vertebrae T8/T9) and at least 3 cm below electrode 4.

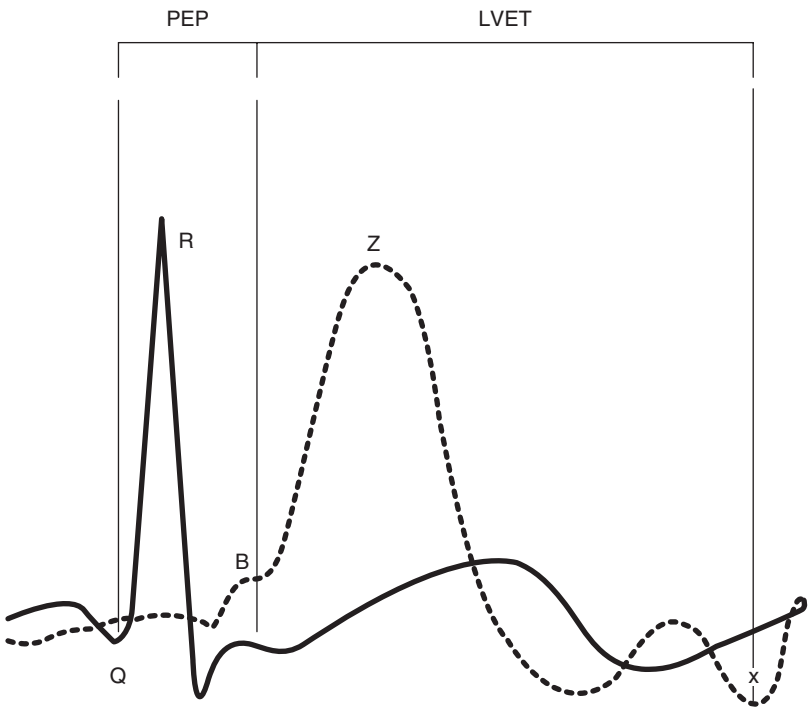


Figure 2.5 Ensembled Δz/Δt and ECG waveforms

Figure 2.5 shows the Δz/Δt waveform superimposed on an ECG waveform. Examining these waveforms together provide critical interrelated inflection points. For example, a chronotropic (time-based) measure of ventricular contractile force is *preejection period* (PEP). This is the time from the left ventricle contracting (Q inflection on the ECG wave) to the aortic valve opening (B inflection on the Δz/Δt waveform). Preejection period is considered one of the purest measures of sympathetic activation. *Left-ventricular ejection time (LVET)* is also a time-based measure determined from the B and X inflections on the Δz/Δt waveform. *Stroke volume*, the amount of blood ejected from the heart on any given cardiac cycle, is a volume-based measure that requires the identification of the B and X- inflections on the Δz/Δt waveform to determine the time when blood is being ejected into the aorta, along with the maximum point of the Δz/Δt waveform on the given cardiac cycle labeled Z^2. The area under this curve is then estimated to determine stroke volume (see formulas below).

Stroke volume provides an estimate of the amount of blood ejected at each beat. The overall indication of how much blood is being pumped out of the the heart at any given time is expressed as *cardiac output* (CO) in liters per minute. Cardiac output is simply the product of SV and HR: (SV × HR)/1000. The metric for stroke

volume is in milliliters, so the product is divided by 1000 to convert to CO which is reported in liters. Because CO is a combination of both heart speed and blood volume pumped by the heart, it is believed to be a measure of cardiac efficiency.

When using impedance cardiography in a laboratory setting, it is important to instruct participants to wear comfortable, two-piece clothing to the experiment. As the bands (or spots) require placement on the torso, directly on the skin, participants are required to lift their shirts to expose their torso. Additionally, placement of the neck sensors might be impeded by clothing that is snug at the neck. A well-equipped lab will keep loose shirts and pants for participants who arrive in clothing that would make attachment of the bands difficult.

Collecting impedance data

Similar to ECG, a common sampling rate for impedance cardiography is 1000 Hz. A low pass filter set at 50 Hz allows for the fine waveform tracing needed to score the waveform. Also, the $\Delta z/\Delta t$ waveform should be amplified to 1 volt at the beginning of the session. Note that by adjusting the amplifier in this way, the amplitude of the Z-point (a major component of SV/CO) will be roughly equated across participants so individual differences in SV and CO will be compromised. The important point here is that because of the variability in sensor placement of the bands (and especially spot electrodes) and individual differences in body morphology, it is highly questionable whether any single measure of SV or CO is valid. Any minor variation in placement of the sensors can dramatically change the amplitude of the $\Delta z/\Delta t$ waveform. Because of this and related problems, researchers typically examine changes (or reactivity) in SV and CO. This is also important in longitudinal studies. Unless there is assurance that the band electrodes were placed exactly in the same location on the body at every assessment, comparing SV or CO over time using a single time point might be highly questionable. However, the chronotropic components of the cardiac cycles that rely only on the X-axis points (PEP, LVET, etc.) are valid as single or repeated point time estimates.

Editing and quantification

Probably one of the greatest challenges for researchers interested in impedance cardiography is how to edit and summarize the data. There are important choices to be made regarding how the data are summarized for editing. One option uses ensembled waveform averages. This method determines the composite or average waveform across a specified time period (typically between 30 seconds and 5 minutes). By "ensemble averaging" the waveforms over time, random noise and movement are removed and a more representative cardiac cycle can be obtained. Another option is to determine blood volume changes on a cycle-to-cycle basis

(see SPR committee guideline paper: Sherwood, Allen, Fahrenberg, Kelsey, Lovallo, & van Doornen, 1990).

In addition to how the data are averaged, there are also several formulas that can be used to estimate stoke volume. The Kubicek equation estimates SV from the derivative of the impedance signal and blood resistivity:

$$SV = \rho \times L^2 / Z_0^2 \times \Delta Z / \Delta t_{max} \times LVET$$

Where: $\rho = 135$ (blood resistivity)

L = distance between electrodes

$\Delta Z / \Delta t_{max}$ = peak amplitude of $\Delta Z / \Delta t$

LVET = left ventricle ejection time (time in ms between B and X)

More recently, other equations have been offered that might be superior to the Kubicek equation. For example, the Sramek-Bernstein estimates SV from the volume of electrically participating tissue scaled according to body surface:

$$SV = \delta (VEPT) / Z_0 \times \Delta Z / \Delta t_{max} \times LVET$$

Where: $\delta (VEPT) = weight_{max} / weight_{ideal} \times (0.17H)^3 / 4.25$

Participant's height, weight, and ideal weight are needed

Regardless of the equation used, one of the most critical decisions involved in scoring impedance data is accurately identifying the *B* point on the $\Delta z/\Delta t$ waveform. Though tremendously time and labor intensive, the technique that assures most accuracy is visual detection of the *B* point. Specifically, *B* should be placed at the beginning of the longest uphill slope before the Z-point.

Applications of impedance cardiography

Cardiovascular responses have been used extensively in the areas of motivation, emotion, and stress. Interests in these measures are further fueled by the possibility that certain patterns or response profiles of CV responses repeatedly experienced over time might be linked to health outcomes. Early work linking type A personality and coronary heart disease examined CV responses as one of the likely mechanisms through which physical health was affected. Specifically, it was theorized that excessive CV responses would create tears in endothelial lining, resulting in greater calcifications and plaque build-up that could possibly initiate ischemic events or strokes. Primarily, CV responses in this context included heart rate (heart period) and blood pressure responses.

A combination of cardiovascular and blood pressure responses is used in research attempting to index challenge and threat states. Though not without its critics (Wright & Kirby, 2003; see also Blascovich, Mendes, Tomaka, Salomon, &

Seery, 2003), this theory attempts to differentiate motivational states using various CV measures, such as PEP, cardiac output, and total peripheral resistance (see below). This theory argues that in *motivated performance situations,* tasks that are active rather than passive and require some cognitive or behavioral responses, profiles of CV reactivity can differentiate approach from defeat orientation (Mendes, McCoy, Major, & Blascovich, 2008). Early work showed that task appraisals in which participants reported having greater resources relative to how demanding they perceived the tasks were associated with greater cardiac responses (shorter PEP [indicating greater ventricle contractility], increased HR and CO, and decreases in vascular resistance – lower TPR). This pattern of CV reactivity was believed to be a marker of psychological states of *challenge*. In contrast, appraisals that showed greater perceived demands relative to resources to cope were associated with comparatively less CO and higher TPR (Tomaka et al., 1993). This profile of CV responses was thought to index *threat* states.

Challenge and threat theory has been tested in a variety of social domains. For example, these indexes have been explored within dyadic social interaction when one member of the dyad is stigmatized. Stigmas were operationalized as physical stigmas (e.g., birthmarks), stigmas resulting from group membership (e.g., race/ethnicity), or socially constructed stigmas (e.g., accents, SES). Across more than a dozen studies, participants who interacted with stigmatized partners were more likely to exhibit threat (i.e., lower CO and higher TPR) than those interacting with non-stigmatized partners. If the results were found only with physiological responses they would have still been intriguing, but in many cases the physiological responses also correlated with other automatic or less consciously controlled responses such as cognitive performance, emotional states, and non-verbal behavior such as freezing, orientation away from the partner, and closed posture (Mendes et al., 2008; Mendes, Blascovich, Hunter, Lickel, & Jost, 2007; Mendes, Blascovich, Lickel, & Hunter, 2002). Also of interest was the lack of correlations between participants' CV responses and their self-reported task appraisals and partner ratings. In contrast with the CV responses, self-reported partner ratings showed a preference for stigmatized compared to non-stigmatized partners, suggesting that deliberate and consciously controlled measures might be more vulnerable to attempts to correct for racial bias (Blascovich, Mendes, & Seery, 2002; Mendes & Koslov, 2010).

In the personality domain, these measures have been used to assess individual's reactions to stressful situations. For example, individuals who score higher on belief in a just world scales (e.g., hard work is rewarded) tend to exhibit greater increases in cardiac and decreases in TPR during stressful tasks than those who score lower on these scales who exhibited lower CO and higher TPR – consistent with threat profiles (Tomaka & Blascovich, 1994). Self-perceptions in the form of level and stability of self-esteem have been explored with these methods as well (Seery, Blascovich, Weisbuch, & Vick, 2004). For participants with high

and stable self-esteem, positive performance feedback resulted in more challenge responses than those with high and unstable self-esteem.

Loneliness appears to result in these profile patterns of CO and TPR (Cacioppo, Hawkley, Crawford, 2002; Hawkley, Burleson, & Berntson, 2003). Cacioppo and colleagues have shown in various settings that individuals reporting higher levels of loneliness are more likely to show lower CO and higher TPR than individuals reporting lower levels of loneliness. This effect has been found in both lab based settings in response to social evaluation, and field studies using ambulatory impedance and blood pressure devices. In the field study, due to lack of ability in determining whether individuals were actually in a *motivated performance situation*, the authors interpreted these profiles as indicating passive versus active coping styles (Sherwood, Dolan, & Light, 1990), with lonely individuals adopting more passive coping styles within the context of their day.

Blood Pressure

Blood pressure, measured in millimeters of mercury pressure (mmHg), refers to the amount of pressure on the vessel walls during the cardiac cycle. Distinctions are made between systolic blood pressure (SBP) and diastolic blood pressure (DBP), which represent peak pressure compared to lowest pressure in the arteries, respectively. Though correlated, these measures may provide unique information and are thus typically both obtained. For example, during stressful or emotionally provocative situations increases in SBP compared to DBP have been identified as part of an adaptive defense patterning (see Brownley, Hurwitz, & Schneiderman, 2000). Systolic blood pressure responses have also been linked specifically to effort expenditure (Wright & Kirby, 2003). Health consequences have been associated with higher SBP and not necessarily higher DBP. For example, Chobanian et al. (2003) reported that elevated SBP, and not necessarily DBP, predicted the development of coronary heart disease.

Although SBP and DBP are often presented separately, one will also find instances in which researchers combine the two in some meaningful way. For example, pulse pressure (PP) is calculated by subtracting DBP from SBP (PP = SBP – DBP). At rest, average pulse pressure is approximately 40 mmHg. During exercise SBP typically increases more so than DBP. Extremes in PP in both directions can indicate abnormalities. When PP is too high this is likely due to artery stiffness, leaky aortic valves, or hyperthyroidism and has been linked to cardiovascular complication (Blacher et al., 2000). Low PP values, typically influenced by low stroke volume, can indicate abnormalities such as congestive heart failure. Another type of averaging is mean arterial pressure (MAP), which is calculated as a type of average (though not an exact mathematical average because DBP is weighted more given its longer time course within a given cardiac cycle), for example: $MAP \approx [(2 \times DBP) + SBP] / 3$.

Mean Arterial Pressure is often used in combination with CO to determine *total peripheral resistance* (TPR), using the formula: TPR = (MAP/CO) × 80. Changes in TPR can be construed as an estimate of the amount of constriction versus dilation occurring in the blood vessels – specifically the arterioles. When the arterioles constrict, less blood can flow to the periphery and this is indicated by an increase in TPR. In contrast, when arterioles expand, or dilate, this allows more blood flow and is indicated by decreases in TPR. Physiologically blood pressure is determined by TPR and CO, but in terms of measuring these parameters, technology is currently superior at measuring BP and CO, and so TPR is calculated from these measures.

Measuring blood pressure

Blood pressure values can be obtained from sensors placed on various parts of the body including the brachial artery (upper arm), radial artery (wrist), or at the finger. It is important to point out, however, that as the distance from the heart is increased the accuracy of blood pressure changes can be reduced. Blood pressure measurements can be obtained using a variety of techniques; here we review four methods for obtaining blood pressure responses.

The first option is the auscultatory method, which consists of temporally stopping blood flow at the brachial artery and listening for sounds ("Kortokoff sounds") indicating blood flow in the arteries – the pressure when blood first begins to flow is systolic blood pressure and the pressure when blood flow sounds stop is diastolic blood pressure. A trained professional uses a sphygmomanometer and stethoscope to obtain BP using this technique.

However, in many cases psychologists want to obtain BP in a less labor intensive way, one that minimizes the self-consciousness that may arise from having one's BP measured. Digital BP machines are relatively inexpensive and fairly accurate (though not as precise as a trained professional using a sphygmomanometer). Again, these BP machines typically require occluding the brachial artery every time a BP measurement is desired. This is not difficult, but could potentially distract participants from the experimental situation. In Figure 2.6a the participant is wearing a Colin blood pressure cuff (Prodigy II) that can be adjusted to take as many as one BP reading per minute. The advantages of this method are that the BP readings are highly valid and reliable. The disadvantage is that the participant's arm is "squeezed" every time – stopping arterial blood flow. Repeated assessments with this technique could artificially elevate blood pressure and/or distract the participant from the study at hand.

The other three options allow the researcher to collect blood pressure *continuously* throughout an experiment. Commercially available machines are manufactured and/or distributed by Biopac (Goleta, CA), Colin Medical Instruments (San Antonio, Texas), and Mindware Technologies (Gahanna, OH). In Figure 2.6b, the participant is wearing a Colin BP monitor (Colin 5000) which uses tonometric technology.

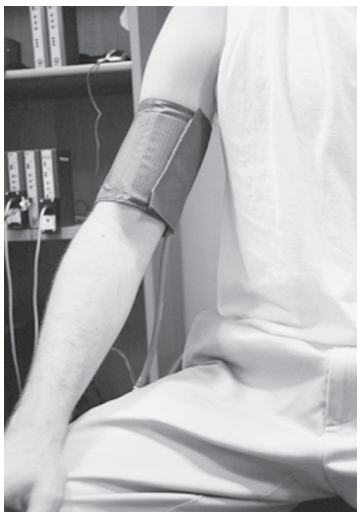


Figure 2.6a Blood pressure cuff over brachial artery (Photos: Christopher Ovies)

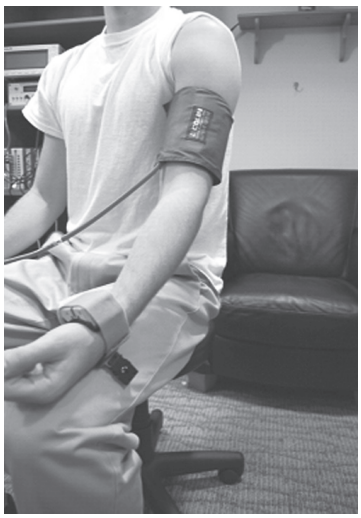


Figure 2.6b Blood pressure device over radial and brachial arteries

Tonometric technology consists of BP measurement from the radial (wrist) artery, and uses a sweep technique, which applies varying force on the artery. This technology can be very sensitive to movement and sensor positioning relative to the heart. Manufacturers recommend putting the arm in a sling so as to position the sensor at heart height and limit movement. For social and personality psychologists who often aim for ecological validity, restraining the arm can be problematic. Fashioning a cradle that will keep the arm and wrist stable throughout the experiment is imperative to obtaining good measurements. Some of the more expensive machines also include an additional brachial cuff BP device to allow for online comparisons from the two sites and can signal the wrist cuff to re-position if the brachial BP responses differ from BP measured at the radial artery.

The third option is the oscillimetric method. Oscillometric technology initially inflates a cuff over the brachial artery and then deflates until the point at which the systolic pressure can be measured, and then keeps a constant cuff pressure. The technology and algorithms used for these machines are proprietary so there is some concern about comparing results across laboratories, and these machines are the most difficult to locate commercially.

The fourth option is the volume-clamp (or Peñaz) method. This method uses a combination of a clamp at a peripheral site, typically the finger, held at a constant volume and a photoplethysmograph to measure blood volume. Blood pressure is estimated by measuring the amount of pressure change in the cuff that is required to keep the volume in the artery at a stable level. The most widely used machine

using this technology is the Finapres, which is no longer available for purchase but is still found in many labs. New machines using this technology can be purchased from Portapres, Finometer, and Biopac.

Applications of blood pressure responses

Social psychologists have used blood pressure to index several psychological states including stress, threat, and effort. Much evidence has been accumulated by Wright and colleagues (see Wright & Kirby, 2003 for a review) supporting their theory of effort mobilization. In this extension of Brehm's motivational intensity analysis, it has been empirically demonstrated that participants' effort increases monotonically with difficulty until the task is perceived as too difficult and then effort is withdrawn. In this model, Wright typically uses SBP as a measure of effort. Although HR and DBP may also follow similar patterns as SBP, SBP is thought to be more closely aligned with effort given its tighter relationship to the sympathetic component of the cardiac cycle (systole).

Skin Conductance

Electrodermal activity (EDA), also known by its outdated name galvanic skin responses (GSR), is a fairly common measure of ANS activity, and one that has a long history in psychological research. Electrodermal activity is a measure of eccrine sweat glands secretions, which are found widely distributed across the body, but are densely distributed in the palms of the hands and soles of the feet. The sympathetic branch of the ANS system innervates these sweat glands, but unlike most ANS responses the neurotransmitter involved in changes is acetylcholine rather than epinephrine.

Electrodermal activity is commonly measured in one of two ways. The first method, *skin conductance,* uses a small current passed through the skin via a bipolar (i.e., dual) placement of sensors and the resistance to that current is measured. The reciprocal of this resistance is *skin conductance.* The second method, *skin potential,* uses no external current and is collected using a unipolar placement of sensors.

In addition to these methods of assessing EDA there are two categories of data quantification that are based on how the EDA data are aggregated. When examining responses to a specific and identifiable stimulus one looks at phasic activity or the *response*. When describing electrodermal activity that is not associated with a specific stimulus onset, but rather changes in EDA over longer periods (i.e., minutes rather than seconds), it is appropriate to examine tonic responses or *level*. Thus, with two methods of collection and two methods of quantifying changes there are four categories of EDA data: s*kin conductance response* (*SCR*),

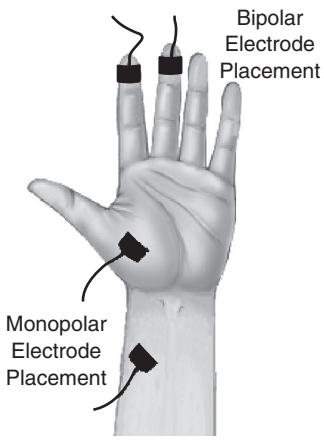


Figure 2.7 Placement of bipolar and unipolar leads for measurement of *EDA*

s*kin conductance level* (*SCL*), *skin potential response* (*SPR*), and s*kin potential level* (*SPL*). Choice of method and quantification should be determined by the specific questions under investigation, which are described in more detail below.

Preparation and recording

To record skin conductance, a bipolar placement of silver–silver chloride sensors are placed on the fingers, palms, or soles of the feet. If finger placement is used it is recommended that the sensors be placed on adjacent fingers (2nd or 3rd fingers; or 4th or 5th fingers) because they will be innervated by the same spinal nerve (Venables & Christie, 1973) (Figure 2.7). Unlike skin conductance recording, skin potential recording requires a unipolar placement in which one electrode is placed on an active site – typically the palm of the hand – and the other sensor is placed on an inactive site, typically the forearm, though any inactive site would work.

Preparation of the skin includes washing with a mild, nonabrasive soap. Use of alcohol-based hand sanitizer or anti-bacterial soap prior to sensor placement is not recommended because the chemicals in them may excessively dry out the skin, resulting in lower levels of EDA and obscure sensitive changes. An electrolyte (either KCL, NaCL) or commercially available conductance cream is then applied in a thin film on the two sensor sites and also in the wells of the sensors. Once the sensors are attached, it is advisable to wait several minutes (typically 5 to 15 minutes) prior to beginning the recording session to allow for the conductance gel to adhere to the skin. Before beginning recording, one should check for sensor sensitivity. Electrodermal activity responds to respiration so the participant can be instructed to take a deep breath and hold his or her breath for a few

seconds. A good connection will be indicated by an increase in SC within 2 to 3 seconds once the breath is initiated.

Editing and quantification

After data collection, electrodermal waveforms should be inspected for movement artifact and electrical interference. Most scoring programs (free or ones available for purchase) have options for editing waveforms that allow a coder to spline, or interpolate, the area of the waveform that is affected by an artifact. This smoothing technique typically removes the influence of artifacts through interpolation by identifying the beginning and end of areas of the waveform that contain an artifact and replacing them with an estimate derived from adjacent areas.

When quantifying EDA to examine tonic levels (SCL/SPL) the decisions for averaging the waveform are time-based, that is, averaging across a specified time period while a participant is at rest and then averaging over a similar time period when a participant is engaged in a task or activity. For example, reactivity values can be calculated in which one-minute of baseline data, typically the last minute or the minimum minute (when EDA reaches its nadir), are subtracted from data quantified in one-minute intervals from a task. These new values represent the change in EDA from resting to a task period. Alternately, ANCOVA or regression techniques could be used in which baseline levels are added as covariates or repeated measures analyses are used to examine changes over time.

A slightly more complicated approach related to quantification is required when examining responses linked to specific stimuli (SCR/SPR). In this case, an identifiable time-locked stimulus is presented to the participant and a *trigger* or stimulus output is recorded online simultaneously with the EDA signal. A minimum threshold value of change needs to be determined so that a change in EDA can be identified as a *response* or not. This threshold can be set at a variety of ranges, but typically the level is set between 0.1 and 1.0 μS (microsiemens). Post-processing of data then allows for an estimate of the change in EDA linked to the specific stimulus. Several measures can be determined from this response: the latency from the stimulus to the initiation of rise time; the time from the initiation of rise time to the peak amplitude; the amplitude; and the time to reach half-delta (Figure 2.8). Half-delta is a time-based measure determined by examining the total magnitude of amplitude increase, divided by two, and then calculating how long it took from peak of amplitude to half of the magnitude increase.

Levels versus responses

The choice of collecting and or scoring data based on examining either levels or responses should be dictated by the research questions and study design. For example, when experimental designs include presentations of specific stimuli in a time-locked

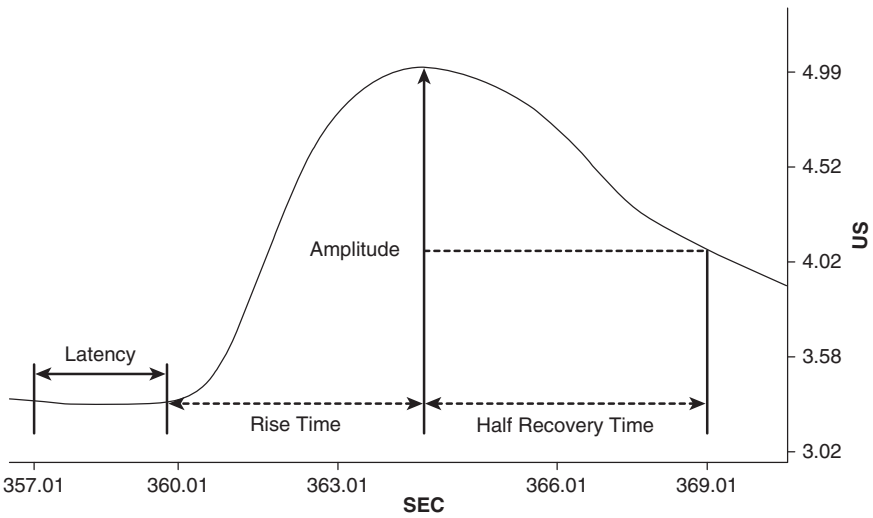


Figure 2.8 Skin conductance response

event-related design (e.g., affective pictures, pictures of members of different racial groups, etc.) it makes sense to score data as responses. When a study design includes events that unfold over time and there are no specific time-locked events (e.g., social interactions, delivering speeches, non-scripted negotiations, etc.) then examining changes in EDA level from a baseline period to a task would be most appropriate. If the decision is made to examine changes in EDA level one can still examine spontaneous responses, but the designation of these responses would be non-specific skin conductance responses (NS-SCR). This measure is typically reported in number of NS-SCRs per minute, with resting or baseline averages ranging from 1 to 3 per minute. This measure could be used as a general index of anxiety, attention, arousal, or linked to different dispositional or clinical factors.

Applications of skin conductance

As previously described, changes in EDA can index general arousal, thus the use of these measures at first blush may seem limited. However, both classic and contemporary uses of these measures show compelling data obtained by looking at electrodermal activity. Indeed, using peripheral measures in the domains of emotion, motivation, and attention has provided important empirical evidence for social and personality psychologists.

For example, skin conductance has been used in the context of emotional disclosure. Pennebaker and colleagues (Pennebaker, Hughes, & O'Heeron, 1987)

examined changes in SCL while participants disclosed traumatic events from their lives. When participants were classified as high disclosures, talking about traumatic events decreased SCL relative to those classified as low disclosures. This finding has been used as a possible explanation of why there are physical and mental health benefits of confession; high disclosures showed lower sympathetic activation than low disclosers.

While getting something traumatic off your chest may be beneficial, forcing yourself to feel good might be detrimental. In another study capitalizing on skin conductance levels, Wegner and colleagues (Wegner, Broome, & Blumberg, 1997) instructed participants to either relax or not while answering questions that were believed to index intelligence (a high cognitive load condition) or answer the same questions that were described as test items (a low cognitive load condition). For participants in the high cognitive load condition instructions to relax resulted in higher SCL than those not instructed to relax. These findings nicely demonstrate the potentially ironic effects of *trying* to relax, which resulted in greater sympathetic activation.

Not surprisingly, ANS responses in general, and EDA activity in particular, are often used in research examining biases and race relations because of the difficulty in obtaining unexpurgated self-reported responses. For example, changes in skin conductance level were used to examine threatening gender environments based on the imbalance of males to females (Murphy, Steele, & Gross, 2007). In this research, male and female participants viewed one of two videos that presented either a gender-balanced group of students or a gender-unbalanced (mostly white males) group of students in the domain of a mathematics and engineering science camp. Changes in SCL from a baseline period to watching the videos were computed. Results showed that women exhibited greater SCL increases when watching the gender unbalanced video than watching the gender balanced video, and male participants did not differ in SCL responses as function of the gender composition of the video. The authors concluded that the gender imbalance was especially threatening for women.

In these examples, EDA was used as a general measure of arousal or anxiety. However, by limiting the context one can increase the inference of EDA changes. For example, in fear conditioning paradigms an electric shock or other aversive stimulus is paired with some unconditioned stimulus while skin conductance responses are measured. In learning phases, the shock or other aversive stimulus is repeatedly linked in time with the unconditioned stimulus. Later the shock is removed and SCRs are examined upon exposure to the unconditioned stimulus. The critical examination is the length of extinction – or how long it takes for participants to no longer show a SCR to the unconditioned stimulus once the aversive element is removed. An exceptionally creative adaptation of this design is found in a Science article by Olsson, Ebert, Banaji, & Phelps (2005). In this study, researchers paired electric shock with ingroup and outgroup male faces.

They argued that individuals might be evolutionarily "prepared" to fear outgroup members and because of this, extinction of the fear response would take longer when the shock was paired with outgroup faces than when the shock was paired with ingroup faces. Indeed, when shocks were paired with outgroup faces compared to ingroup faces, SCRs persisted longer and were of greater magnitude in the extinction phase. In this example, SCRs could be interpreted as fear responses because the context was constrained to a fear eliciting (shock) situation.

SC changes may index emotional responses even prior to the conscious awareness of that emotion. An elegant example of the possibility that physiology may provide information regarding emotional and motivational responses before conscious awareness is provided by Bechara and colleagues (Bechara et al., 1997 cf. Maia & McClleland). These investigators measured SC changes while participants learned decision rules for a card task. Although self-reported indications related to "hunches" regarding decks that were associated with more gain than loss cards developed by trial 50 for control participants (i.e., non-patient), SC changes in anticipation to the loss decks occurred typically by the 10th trial, which preceded conscious awareness by approximately 40 trials. That is, SCRs suggested an intuition of an impending loss prior to participants' conscious awareness of the intuitions they were developing.

Data Editing

There are many software programs that are free or available to purchase. However, the most critical feature of any scoring program is the person who is scoring or processing the data. Many software programs have algorithms to find various points on the waveform or to identify artifacts, but some visual inspection is typically required.

The major concerns of data editing are the reliability and validity of the editing. To increase reliability of data editing, a subsample of data should always be re-scored by a different person to determine consistency. For some measures, which involve less editing or fewer "gray" areas in judgment, one should expect near perfect reliability – for example, IBI, RSA, blood pressure responses, skin conductance responses. For other measures in which there is some subjective judgment, like at what point does the B inflection occur on the $\Delta z/\Delta t$ wave, one should look for very high reliability, but not necessarily perfect reliability. We can achieve reliability of different data editors with $\alpha > 0.95$ for measures such as PEP, SV/CO, LVET. Research assistants who are very conscientious and patient and who have good pattern detection skills make the best data editors.

Of course just because a group of research assistants are reliable does not mean that they are producing valid data. We suggest several guidelines to determine the quality of the data while scoring with a focus on the validity of the measures. One guiding rationale is *physiological plausibility*. Each measure has a range of

responses that are plausible given the physiological marker. In addition to plausibility of any single measure, there is also plausibility given a constellation of multiple, but related measures. Table 2.1 shows plausible ranges of PEP, HR and LVET (left ventricle ejection time – time from the aortic valve opening to the time that it closes). These relations demonstrate that when the heart is beating faster, we expect decrease in PEP and LVET. These ranges are not presented as the only possible ranges that could occur, but rather general guidelines to determine if the data are typical or not. When scoring or examining data one should be aware of general ranges in which these measures are related to each other.

Designing Studies

One never wants to be in a position in which he or she designs their research questions around their methodology, but as is the case with all methodologies there are some constraints depending on the measures targeted. The good news is that there are many options within electrophysiological recordings to get around some of the constraints that many methods do not enjoy yet – for example, as of this writing fMRI still has to be conducted while supine in a large magnet. Here we review issues related to designing experiments and how the method can be adapted to the question at hand.

Timing

Researchers need to determine whether their study is time-based or event-based. Simply, the difference between these options is that in a time-based design, the unit of measurement for ANS responses is a time window (30 seconds, 1 minute, 5 minutes, etc.), whereas for an event-based design the unit of measurement is triggered by an event and then the length of the event can be defined by either the total length of the event or some pre-specified time window. Time-based studies require the experimenter to determine a priori the length of the experiment – how long is baseline, different tasks, and a recovery period. Once this is determined a time-based study will then obtain physiological data in bins, which means a pre-specified time period for which ANS responses are collected and labeled. For example, 5-minutes of rest at the beginning of a study would require the experimenter to obtain a 5-minute bin of data. One could still break down the bins into smaller units to score the data, for example 30 seconds or 1 minute, but the smallest collection bin would set the largest time unit for scoring.

Event-based designs collect ANS responses based on a *trigger* that indicates the beginning of a phase of the experiment. In these designs, physiological data tends to be collected continuously throughout the experimenter with no bins identified. Instead, computer generated triggers are inserted into the acquisition software that is collecting the physiological data (or an experimenter might manual insert triggers

Table 2.2 Plausibility of physiological ranges: HR, PEP, and LVET

HR (bpm)	*PEP (ms)*	*LVET (ms)*
40–60	100–140	300–450
60–80	90–130	250–400
80–120	80–120	250–350
100–120	70–100	200–300
120 +	<80	180–300

with a key press at certain events). When collecting data for an event-based experiment to score or average the data for a specific event requires both the trigger that initiated the event and either a second trigger that indicates the end of the event or a known time interval that indicates the length of the event (e.g., 33 seconds from the initiation trigger).

Study context

There are several critical design considerations that influence choice of ANS measures, how the data are collected and quantified, and, most critically, how the data are interpreted (see Table 2.2). When incorporating ANS measures in experiments, one of the first decisions necessary involves the context in which ANS responses are collected. As described earlier, many of the inferences that can be drawn from the physiological responses are context bound. In fear conditioning studies, for example, SCRs are often used as the primary measure of fear (e.g., Olsson et al., 2005). However, SCRs are by no means universally accepted as indexing fear responses. Indeed, SCRs can result from strong positive emotion, anxiety, deception, attention, and other psychological factors that are certainly distinct from fear. So it is important to know if a physiological response is believed to be context bound or context free. As the context is more constrained the inference level is likely to increase, though there is little empirical data on this topic.

One of the critical context distinctions when examining ANS responses is the extent to which the participant is engaged in an active versus passive task (Obrist, 1981). Active tasks are ones in which some response is required by participants, as opposed to passive tasks that are simply situations in which participants experience some event without having to necessarily respond in some instrumental way. This distinction is critical because in many cases ANS changes are functional and changes in ANS are due to the required needs of a task rather than the psychological change brought on by the situation. For example, giving a speech requires modulation of respiration to produce vocal tones and often postural changes occur to allow for projection in vocalization, which can influence ANS responses that have nothing to do with stress, emotion, or motivation. In addition, many ANS patterns or profiles are thought to index psychological states from active situations and not passive ones. Challenge and threat motivational states, for example,

are thought to occur only in active situations (Blascovich & Tomaka, 1996) and not passive ones. So watching a scary movie might be terrifying, as would be giving a talk to a room filled with people who you knew disagreed with you, but only in the latter case would the ANS responses yield a validated pattern associated with threat.

Participants' health

Recruiting participants for psychophysiology studies poses some challenges. Depending on the response of interest, there might be some health conditions that should be considered exclusionary. Of course, when interest is in either cardiovascular responses or heart rate variability people with heart conditions, abnormalities, pacemakers, or cardiac altering prescriptions (like beta-blockers) should be excluded.

There may be occasions when an abnormality is detected on the ECG, and what to do when this is detected is actually a matter of debate (see Stern, Ray, & Quigley, 2003). One perspective is the fact that non-medical professionals informing participants that there might be some abnormalities in their ECG can cause undue distress if proven wrong. The other perspective is that abnormalities can be detected with ECG waveforms and that an informed opinion could be beneficial to participants so one should inform. Decisions to report should be guided by your local IRB and the quality of one's knowledge of ECG abnormalities. For several years, we received advice from a cardiovascular surgeon when concerned about possible cardiac abnormalities. Forging a relationship with a medical professional might be critical if an IRB or funding agency wants you to report abnormalities that are detected to participants. Importantly, though, undergraduate research assistants with limited experience and graduate students just starting should not make these decisions, but the lab should have some plan for how to deal with these possible situations. There are no requirements to report unusual signals, which may, or may not be, abnormalities.

Individual response stereotypy

For social psychologists another potential source of difficulties with participants is individual response stereotypy or the idea that for some individuals, regardless of the situation, ANS response will not be modulated as predicted by the situation. For example, some individuals are thought to be chronic vasoconstrictors and regardless of the situation will show constriction rather than dilation in their arteries and arterioles in any change from homeostasis. There is considerable disagreement in the literature regarding the percentage of individuals who respond without psychological modulation, but it is something that could add error and reduce the ability to detect differences based on the experimental manipulation. Certainly

older participants are more likely to have sluggish responses and tend to have more individual response stereotypy than have modifiable responses. Similarly, overweight individuals also might show less psychological modulation.

Situational response stereotypy

Parallel with the idea that some individuals respond in similar ways without the influence of the social setting, some situations are thought to bring about similar responses without individual modulation. One of the most obvious situations is the startle reflex in which sound or visual presentations occur at such high decibels or lumens that the blink reflex occurs for everyone. At lower levels of sound, for example, psychological modulation can occur, so only at intense levels is the startle response universal.

Future Directions

One of great advantages of ANS recording that has been underutilized is examining the dynamic nature of changes in ANS responses as a result of moment-to-moment changes in experience. In many cases, psychophysiologists spend a great amount of time and effort reducing their data to a reasonable number of time epochs and critical responses. However, statistical techniques like hierarchical linear modeling (HLM) and time series analyses allow researchers to model temporal changes in a more finely grained fashion than ever before (Vallacher, Read & Nowak, 2002). An additional benefit of these online responses is that they do not require a conscious assessment of what one is thinking or feeling. Thus, responses can be viewed as relatively automatic and less consciously controlled than online subjective reports obtained with rating dials.

ANS responses are not limited to lab-based designs. Advances in ambulatory monitoring allow for responses collected continually throughout a person's daily life and coordinated with experience sampling techniques. Ambulatory monitoring of ANS responses presents infinite possibilities for social and personality psychologists, not to mention those who intersect with public health, clinical science, and organizational behavior. The possibilities are endless and limited only by researchers' imagination, knowledge and resources.

3

Electromyography and Startle Eyeblink Modification

All behavior is a product of the coordinated contractions of some of the several hundred muscles in the human body. Talking, smiling, kissing, pressing computer keys, aggressing, discriminating, purchasing, voting, gesturing, leading – these are among the many actions studied by social psychologists everyday, and each is ultimately mediated by the somatic nervous system. Surface electromyography (EMG) is a noninvasive measure of muscular activity involving the attachment of small sensors to the skin on top of a muscle region of interest.

As the underlying muscle begins to receive stimulation from a motor neuron at the motor endplate (situated near the middle of muscle), individual muscle fibers begin to contract as a muscle action potential propagates bidirectionally from the endplate. The electrical activity associated with these action potentials permeate through the surrounding tissue and reach the skin's surface where the EMG electrodes can conduct the signal to an amplifier. What makes EMG particularly useful to the psychologist is that the measured EMG activity corresponds directly to the size of the contraction of the underlying muscle. Due to space limitations, this chapter will not consider the physical basis of EMG further, but for more details on muscle physiology and EMG, the reader should consult Tassinary, Cacioppo, and Vanman (2007) and Hess (2009).

What is covered here are some of the main methodological concerns and techniques involved in the recording of EMG, particularly with respect to the types of research conducted in social psychology. The chapter begins with a discussion of issues that are relevant when a researcher is considering using EMG in a study. Specific recommendations are made regarding laboratory procedures and the specialized equipment required for EMG research. Finally, after reviewing how EMG is recorded during the experimental session, the chapter concludes with a consideration of quantification and data analytic issues. Throughout the chapter various aspects of EMG including start eyeblink modification are illustrated via a hypothetical study of the perception of trust.

Although EMG can be recorded from nearly any muscle region on the body, this chapter focuses on the recording of EMG from the face. Contemporary

psychology researchers use EMG primarily to measure the activity of muscles that underlie facial expressions of emotion. In addition, they also use EMG muscle activity recorded from the eyelid to measure startle eyeblink reflex modification. But why bother going about recording EMG activity in the first place? The point of the next section is to present the reader with some of the unique advantages that EMG provides for a researcher in social psychology.

The Promise of Facial EMG and Blink Modification

An increased interest in emotion in recent decades has made the face an important source of data about emotion and affect (Kappas, 2003). Scientific interest in the face, however, actually began in the early nineteenth century. For example, Charles Bell (1844) and Guillaume Duchenne de Boulogne (1862/1990) set out to map out the configurations of the facial muscles that underlie various emotional states. Charles Darwin devoted most of his *The Expression of Emotions in Man and Animals* (1872) to covering the evolution of facial expressions, referring extensively to the work of his predecessors as providing support for his notions (Hess & Thibault, 2009).

During the twentieth century, psychologists' interest in the face as a "leaky channel of expression" waxed and waned. Most research focused on overt changes – clear, visible signs of anger or happiness, for instance – that led to the development of sophisticated scoring methods of facial movements, such as the Facial Action Coding System (FACS; Ekman & Friesen, 1978; Hager, Ekman, & Friesen, 2002) and the Maximally Discriminative Affect Coding System (MAX; Izard, 1979). These systems require extensive training and hours of coding to derive objective, quantitative measures of various facial expressions that appear in video recordings.

Facial EMG

The recording of EMG provides another objective method to measure facial muscle activity that underlies emotion expressions. The recent growth in the use of this method can be traced to a now "classic" EMG study (Schwartz, Fair, Salt, Mandel, & Klerman, 1976), which involved instructing depressed and non-depressed participants to imagine a typical day in their lives while EMG was recorded over four facial muscle sites. Although the non-depressed participants exhibited a pattern of facial EMG activity that was similar to what happened when they were asked to imagine a happy event (i.e., less frowning – decreased corrugator activity), the depressed participants exhibited a pattern for a typical day that was similar to one in which they imagined a sad event (i.e., more frowning – greater corrugator and depressor anguli oris activity). This study was a promising example of how one could measure facial expressions of emotion without extensive video equipment and intensive labor.

Since the publication of Schwartz et al.'s study, most facial EMG research in psychology has focused on two muscle sites (see Figure 3.1), the zygomaticus major (the muscle in the cheek that pulls up the lip corner) and the corrugator supercilii (the muscle above each eye that pull the brows together), because these facial muscles typically exhibit increased activity during times when the participant later reports having experienced positive or negative affect, respectively (Cacioppo, Petty, Losch, & Kim, 1986). In addition, some researchers also attach electrodes over the orbicularis oculi region to record activity associated with "Duchenne" smiles, which tend to form wrinkles or crow's feet at the outer corners of the eye (Ekman, 1992; Hess & Blairy, 2001), although orbicularis oculi activity does not always accompany zygomaticus activity associated with positive affect (Fridlund, 1994; Schneider & Unzner, 1992). Facial EMG can also assess covert muscle activity – low levels of muscle contraction which do not necessarily

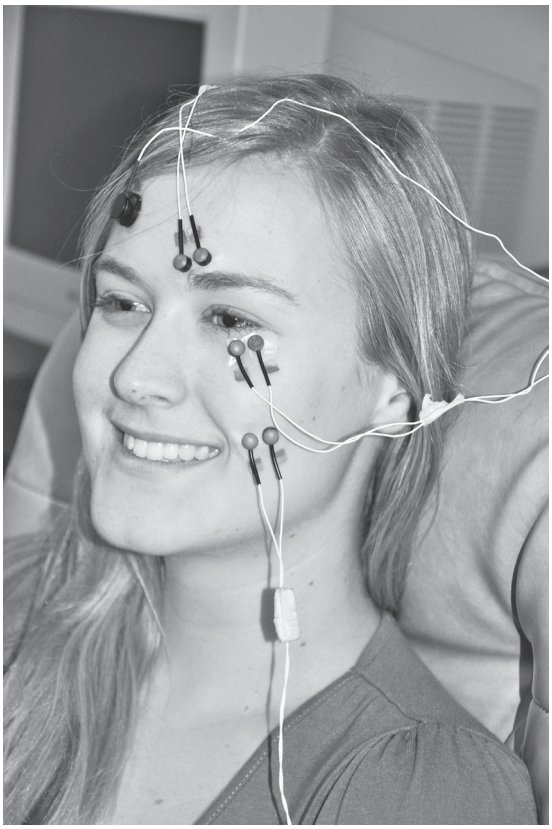


Figure 3.1 Electrodes placed over the zygomaticus major (cheek), corrugator supercilli (brow), and orbicularis oculi (eyeblink) regions. The electrode on the right forehead is being used as a ground (Photo: Eric Vanman)

lead to overt facial movements that can be observed by FACS, for example (Tassinary & Cacioppo, 1992).

Although facial EMG offers a less time-consuming method to quantify the activity of facial muscles, compared with methods involving systematic coding of facial expressions, it is still a relatively unobtrusive measure. Once the electrodes are attached, participants often report being unaware of them. This means that participants do not need to be interrupted while they are performing experimental tasks. Affective and cognitive responses can be continuously monitored throughout the testing session. And, although EMG can index these responses, participants are often unaware of their responses or are possibly unwilling to report those of which they are aware (e.g., Hazlett & Hazlett, 1999; Tassinary, Orr, Wolford, Napps, & Lanzetta, 1984; Vanman, Paul, Ito, & Miller, 1997).

Vanman et al. (1997), for example, recorded facial EMG while White American participants imagined cooperating with African American or White partners in various scenarios. Participants reported more positive feelings when imagining working with Black partners than they did when imagining working with White partners. Patterns of facial EMG, however, indicated a different affective response. Specifically, participants exhibited greater activity over the zygomaticus major region when they imagined working with a White partner compared to a Black partner. EMG activity over the corrugator supercilii region was greater when they imagined working with a Black partner compared to a White partner. The results suggested that the White participants in that study harbored more prejudice against African Americans than they actually reported. Subsequent research has shown that the racial bias revealed by EMG is related to general racial attitudes and predicts discriminatory behavior as well (Brown, Bradley, & Lang, 2006; Dambrun, Despres, & Guimond, 2004; Vanman, Saltz, Nathan, & Warren, 2004). Thus, an advantage to using facial EMG is that it is difficult for the participant to control, particularly if he or she is not aware of what is being measured, and thus it is less susceptible to some of the biases and distortions to which self-report measures are vulnerable (Tassinary et al., 2007).

Startle eyeblink modification

The startle eyeblink reflex is most often recorded as muscle activity from the lower lid (orbicularis oculi inferior) in response to a startling stimulus, such as a loud noise. This reflex occurs very quickly in humans and is mediated by relatively simple neuronal circuits (Lee, Lopez, Meloni, & Davis, 1996). However, it is the modification of the startle reflex by an immediately preceding foreground stimulus, such as the presentation of a photograph that is of particular relevance here.

One line of startle modification research has demonstrated that the reflex can be augmented or attenuated as a function of attention (Anthony, 1985; Filion, Dawson, & Schell, 1993; Hackley & Graham, 1983; Lipp, Siddle, & Dall, 1997;

Simons & Zelson, 1985). When attention is directed away from the startle stimulus by the foreground stimulus, the startle reflex is smaller at long lead intervals (i.e., the time between the foreground stimulus onset and the startle onset is greater than 1 second). For example, acoustic startle probes resulted in more blink attenuation when young infants viewed interesting compared to dull visual stimuli (Anthony & Graham, 1983). Other studies have shown opposite effects of attention. For example, regardless of sensory modality, when participants are instructed to attend to a foreground stimulus (i.e., a tone), the startle reflex is facilitated at long lead intervals, compared to when startle probes are presented during foreground stimuli that participants are instructed to ignore (Lipp et al., 1997). In sum, it appears that startle eyeblinks are attenuated for foreground stimuli that the participant finds especially salient or interesting, but are augmented for stimuli towards which the participant is deliberately directing his or her attention (see Filion, Dawson, & Schell, 1998, for a comprehensive review).

Another line of research on startle modification has focused on how the affective properties of a stimulus such as a photo of a snake, or an attractive nude, either augment or attenuate the startle reflex (e.g., Bradley, Cuthbert, & Lang, 1993; Stritzke, Patrick, & Lang, 1995; Vanman, Boehmelt, Dawson, & Schell, 1996; Vrana, Spence, & Lang, 1988). The amplitudes of startle blinks that occur when the participant is viewing a negative stimulus are generally greater than when the stimulus is neutral or positive, especially when the participant has had sufficient time to look at the stimulus before the startle probe is presented (i.e., a lead interval of 800 ms or longer). For those stimuli that represent clear instances of threat (e.g., attacking animals and threatening faces), individuals respond with increased autonomic activation and heightened somatic activity (Lang, Bradley, & Cuthbert, 1990). In particular, the amplitude of the startle reflex, a defensive reflex that apparently serves a protective function throughout the body, is hypothesized to be greatest when the aversion system is most active. Thus, a sort of "affect match" occurs between the foreground stimulus and the startle stimulus. In contrast, when the individual is motivated to approach the stimulus (e.g., attractive faces and cute puppies) the amplitude of the startle is hypothesized to be smaller because of an affect mismatch.

Startle modification has not been as widely used in social psychology as in other subdisciplines, but there has been recent interest in it as measure of prejudice. That is, viewing pictures of outgroup members for which an individual holds antipathy should be accompanied with facilitation of the startle response, in comparison to viewing pictures of ingroup members (see also Guglielmi, 1999). This hypothesis has mixed support in studies that used startle modification as an index of racial prejudice. For example, in one study (Amodio, Harmon-Jones, & Devine, 2003), White participants, who had completed measures of prejudice towards Blacks, viewed White, Black, and Asian photos in a startle modification paradigm. At the early lead interval (400 ms), the most prejudiced participants

exhibited more inhibited startle to Black faces than they did to White faces, whereas at the late lead interval (4000 ms) the more prejudiced subgroups evidenced more facilitated startle to Black faces than to White faces. Interestingly, there were no ingroup–outgroup differences when participants viewed photos of Asians. Similarly, Phelps et al., (2000) included a startle modification measure in a study in which Whites also viewed pictures of Blacks and Whites while in an fMRI scanner. Although there were no significant startle modification effects when probes were presented between 2 and 4 s following picture onset, startle modification differences were correlated (r=0.56) with differences in the activity of the amygdala (a subcortical structure that has a major role in fear). Finally, Brown, Bradley, and Lang (2006) presented White and Black participants photos of Whites and Blacks in either positive or negative contexts. Although startle modification was affected by the emotional valence of the photos, the race of the subjects in the photos had no effect on startle. It is important to note that affective modification of startle effects typically occur when the foreground stimuli are highly arousing (e.g., attractive nudes versus a bloody hand) or in arousing contexts, such as when there is a possibility that one will receive a shock (Bradley & Lang, 2007). Therefore, in intergroup situations where contact with the outgroup is not characterized by threat or other strong emotions, stimuli representing the outgroup may be less likely to elicit effects of affective modification of startle, and be more sensitive to the attentional qualities of the stimulus.

Regardless of one's reason to use measure the startle reflex modification as index of attentional or affective processes, this methodology offers some advantages over self-report measures and facial EMG more generally (Blumenthal & Franklin, 2009; Dawson, Schell & Böhmelt, 1999). First, the startle reflex and its modification by psychologically-relevant variables are automatic, and therefore provide information that, even more so than facial EMG, is less susceptible to response bias and the ability of participants to make reports. Startle can also be studied across the lifespan and can be measured across wide range of animals. Its underlying physiology, including its neural basis, is well understood, especially compared to other physiological measures. Finally, the fact that startle probes can be presented at virtually any lead (e.g., 50 ms following the onset of the foreground stimulus) makes it a useful tool for investigating both early and late psychological processes (Filion et al., 1993; Putnam & Vanman, 1999).

EMG Activity as an Index of Stress

So far this chapter has focused on the use of EMG to measure affective processes. EMG is also widely used by many researchers in psychology, particularly by health psychologists and those interested in biofeedback, as a measure of general muscle tension, usually in the forehead (the frontalis) or the back (the trapezius). The idea

that there is coherent tensional factor can be traced back to Woodworth and Schlosberg (1954), who suggested that EMG activity, particularly in the neck region, could be a unique indicator of overall activation. Consistent with this notion, Eason and White (1961) found that the general level of EMG activity recorded at multiple sites (including the splenius, the upper and lower trapezius, the deltoids, and biceps) increased as participants exerted more effort on a variety of vigilance tasks (the performance of which did not directly depend on the activity of those muscles). Similarly, when participants performed complex reaction time tasks that demanded continuous attention but little physical activity, increased attention-related EMG activity was observed over the frontalis and upper trapezius regions (Waersted & Westgaard, 1966). Similar findings, in fact, provide a rationale for the recording of frontalis and trapezius EMG activity that typically occurs during biofeedback therapy designed to increase relaxation. Interestingly, the evidence for a coherent tensional factor is sparse (Fridlund, Fowler, & Pritchard, 1980; Tassinary et al., 2007). In fact, over 50 years ago, Meyer (1953; Meyer, Bahrick, & Fitts, 1953) argued that eyeblink rate provided the best overall measure of generalized tension. And startle reactivity, whether measured as EMG over the forearm extensor or the orbicular oculi regions, has been found to be related to individual differences in generalized anxiety and stress (Britt & Blumenthal, 1992; Davis, Malmo, & Shagass, 1954; Grillon & Davis, 1995).

Recording "Pre-behavioral" Activity

As already mentioned, EMG can be used to measure muscle activity at the pre-behavioral level. That is, even in the absence of an overt movement (e.g., a smile), sub-threshold muscle activity can be recorded by the placement of surface electrodes over the muscle region. In fact, prior to the more recent emphasis on using EMG to measure the muscles that comprise facial expressions of emotions, psychologists had for several decades used this methodology to measure covert activity associated with task performance. In one illustrative study, participants were asked either to lift or imagine lifting weights (Shaw, 1940). Naturally, EMG activity was greater when participants actually lifted heavier and heavier weights, but this same trend was observed when participants simply imagined lifting those same weights. This finding has been replicated and extended several times since (e.g., Bakker, Boschker, & Chung, 1996).

EMG has also been used to measure silent speech processing. For example, McGuigan and Bailey (1969) instructed participants to read, memorize prose, listen to prose, listen to music, or attentively listen to nothing, while EMG activity was recorded over perioral (i.e., lips, chin, tongue) and forearm flexor regions. The findings from this study, and those from similar research (see McGuigan, 1978, for a review), indicated that perioral EMG activity was associated with

silent reading. In a later study, Shimizu and Inoue (1986) recorded perioral and non-oral EMG activity during sleep. When participants were awakened and asked about their dreaming, 88% of those who had shown increased perioral activity just prior to awakening reported they had been speaking in their dreams, whereas only 19% of those who had no increased perioral activity just prior to awakening reported that they had been speaking. And, perhaps more relevant to social psychology, perioral EMG was used by Cacioppo and Petty (1979, 1981) to measure cognitive processing during the presentation of counter-attitudinal messages.

In sum, EMG is used in several ways by researchers in psychology. In addition to what has been mentioned, EMG has also been used to quantify muscle tension in ergonomics (e.g., Delisle, Lariviere, Plamondon, & Salazar, 2009; Peper, Wilson, Gibney, Huber, Harvey, & Shumay, 2003), to study mimicry of hand, arm, and even oral cavity movements (Berger & Hadley, 1975; Cattaneo et al., 2007), to measure incipient activity that proceeds the actual response in reaction time tasks (e.g., Hasbroucq, Burle, Vidal, & Possamaï, 2009; McGarry & Franks, 1997), and to study the muscles that maintain posture and coordinate gait in highly skilled acts (e.g., Trepman, Gellman, Solomon, Murthy, Micheli, & De Luca, 1994).

The Challenge of Emotion Specificity

A major limitation to facial EMG and startle eyeblink modification as measures of affective responses is that both methodologies rely mainly on a dimensional approach to emotions, such that both largely assess positive–negative affect and not more discrete emotions. Hess (2009) discussed a few problems in using facial EMG to identify specific emotional expressions. First, it is not certain that people reliably make clear, well-defined, facial expressions that correspond to well-defined, specific emotions. In actuality, spontaneous facial expressions are often weak and incomplete (Motley & Camden, 1988). Second, Hess noted that EMG researchers are usually limited to recording four or five pairs of electrodes at a time. Having more pairs than this on the face becomes intrusive and creates more discomfort for the participant. This limitation in electrode pairs means that the number of possible muscle sites (and expressions) is also limited to a small number. In addition, the use of surface electrodes allows for cross-talk between muscle sites that can be hard to differentiate. Hess noted that the masseter, a muscle primarily involved in closing the mouth during chewing but also associated with anger (when clenching the teeth), is a much stronger muscle than the zygomaticus major, yet the recording sites for the two muscles are quite close to each other. It is therefore possible that an increase in zygomaticus activity might be observed during anger, not because one is smiling when angry, but because the

masseter is actually contributing to the signal being recorded. Finally, the same muscle can be a component in several different facial expressions. Hess and Blairy (2001), for example, found that corrugator supercilii activity occurs during both angry and sad facial expressions. Overall, the specificity of facial EMG (and startle eyeblink modification) in measuring discrete emotions is rather limited when compared with a more comprehensive scoring system like FACS. Facial EMG can still be useful in this regard, as long as the researcher can limit the range of possible expressions to be investigated (e.g., Vrana, 1993; Wolf, Mass, Ingenbleek, Kiefer,Naber, & Wiedemann, 2005).

Other Limitations

The use of EMG has other limitations as well. First, to reduce the influence of muscle activity not associated with the researcher's interest and research question, participants must remain relatively still through testing. This means that they usually (a) will have to remain seated throughout all tasks, (b) cannot move their heads to look at things in the testing room, thereby restricting the visual field, (c) must refrain from talking, particularly when facial EMG is recorded, and (d) make limited movements with their hands and arms. Startle modification has an additional problem because the elicitation of the startle response is aversive. Some participants may complain about the repeated bursts of the loud noise (typically presented at 105-dB, which is about the same level of noise as when one stands near a power mower or a motorcycle). Participants, in the middle of the testing session, have been known to remove the headphones when the startle noises have been presented. Such restrictions on what and whom can be tested with EMG means that some types of research are not possible with current technology and methods.

One additional caveat applies to nearly all types of psychophysiological research, and is certainly relevant here. It is nearly impossible to make inferences about muscle activity, and its underlying psychological significance, without the presence of discrete stimuli to which the participant responds. For example, the use of facial EMG to index whether or not the participant is in a good mood is nearly pointless if the researcher intends simply to make recordings for the first five minutes of the study while participant is resting. A better solution would be to ask the participants to imagine an event that occurred earlier that day, similar to the method used by Schwartz et al. (1976). Similarly, using startle modification to index affect while a participant watches a scary film will be rather meaningless unless the startles are presented during specific events in the film (e.g., as the actor is about to open a door). Of course, spontaneous activity (i.e., EMG responses that occur in the absence of a known stimulus), such as increased blinking or brief smiles, can be informative, but they would also require some

additional probing or control of the context to be meaningful (Cacioppo, Martzke, Petty, & Tassinary, 1988).

Implications of above for the Hypothetical Study of Trust

As stated above, throughout this chapter, the hypothetical study that serves to illustrate some of the issues covered is one designed to measure affective responses as participants view photographs of trustworthy and untrustworthy faces. Trust involves brain structures associated with emotion (Adolphs, 2002) and the perception of facial expressions plays a role in judgments of trust (Krumhuber, Manstead, Cosker, Marshall, Rosin, & Kappas, 2007), so this hypothetical study examines whether trust elicits affective responses that can be measured by both facial EMG and startle eyeblink modification. Although most of the prior research using facial EMG and startle have investigated research questions in which strong emotional reactions are elicited in the experiment (e.g., the presentation of gory photos, films of medical procedures, the recall of autobiographical material), both methodologies have also been used to measure low-level affect associated with attitudes, intergroup bias, and other social stimuli.

This study involves presenting still photos of faces for a brief period, which are accompanied by a computer-controlled event marker to establish when the photo was presented. For the facial EMG trials, activity from the zygomaticus, corrugator, and orbicularis oculi sites are recorded while the participant simply looks at the photo. On some trials, however, startle probes are presented at some time interval after stimulus onset. This study focuses on probes that occur at both early (less than 500 ms) and late (greater than 1 second) positions. Only the EMG activity recorded from the orbicularis oculi site is analyzed for startle blinks. Note that the large startle response typically elicited by a startle probe would interfere with the EMG recorded at the zygomaticus and corrugator sites, so any design involving those sites would require sufficient presentations of the stimuli that did not include the presentation of the startle probe. In addition, to increase the reliability of the measures, the design includes six presentations of each stimulus type for each of the relevant measures. Thus, six trustworthy faces and six untrustworthy faces are presented during which no startle probes are included, six of each with startle probes presented early, and six of each with startle probes presented late. The within-subjects design of this kind of study, which includes multiple presentations of each level of the independent level, may not be appropriate for research in which there may be concerns about relatively fast habituation or stimulus carry-over effects.

Equipment and materials

Because facial EMG requires the storage of both permanent equipment (e.g., amplifiers, computers, electrodes) and consumables (e.g., collars, gel, paperwork),

a lab should have sufficient surface areas to store equipment, as well as adequate work space to complete preparatory tasks (e.g., attaching electrode collars to the electrodes and applying gel). In addition, when a testing session is finished, clean up is much easier when a sink is nearby. Sometimes participants like to wash their face before leaving the lab, and electrodes need to be washed (and possibly soaked). If only one small room is available for testing, it is certainly possible to conduct the study without a sink and lots of counter space, but the researcher will probably need to be more creative in how to best use a small space, perhaps by using mounted cabinets and rolling tables.

Another concern when considering potential laboratory space is that EMG signals can be obscured by noise from many sources, such as external electrical wiring in the walls or ceilings. The main problem is that such the frequency (60 Hz in North America, 50 Hz generally elsewhere) and amplitude characteristics of electrical noise overlap with those used in EMG. In addition, televisions and computer monitors also tend to generate high-frequency electrical noise. Therefore, careful consideration should be given when designing EMG lab space to minimize electric noise wherever possible by placing equipment as far as practical from these other electrical sources and/or by using shielding techniques (Bramsley, Bruun, Buchthal, Guld, & Petersen, 1967; Marshall-Goodell, Tassinary, & Cacioppo, 1990). Participants may also be affected by voices and other sounds that emanate from outside the testing room, which can create additional artifacts in the physiological recordings, so lab space that is acoustically isolated from the rest of the building is also preferred.

Finally, a key (and often forgotten) feature of any psychophysiological experiment is that the participant is always being studied in some sort of social context, whether as result of a social manipulation (e.g., the use of a confederate or the presentation of faces) or when one simply considers the whole testing session as a social interaction between the researcher and the participant (Cacioppo & Petty, 1983). The effects of being observed on social behavior, for example, are well known, but the mere observation of a participant simply listening to tones can have even consequences on psychophysiological responses (Cacioppo, Rourke, Marshall-Goodell, Tassinary, & Baron, 1990). Facial EMG activity, in particular, can be affected by the presence of another person – even when the participant is alone but believes that another is viewing the same stimulus in different room (Fridlund, 1991; Hess, Banse, & Kappas, 1995). Thus, whenever possible, the experimenter and the participant should be in separate rooms throughout the experiment, and the sense of 'being observed' should be kept to a minimum through the use of concealed cameras or observation glass. One final consideration is the extent to which the testing room's appearance heightens or lessens any anxiety that the participant might be experiencing because of the nature of the research. The concealment of wires and cables, the use of comfortable furniture, and the dimming of lights are more likely to reduce apprehension about the fact that the electrodes are going to be attached to the participant's face.

Specialized equipment

Several manufacturers provide EMG equipment at reasonable prices. Nearly all use modular systems, in which there is some sort of base amplifier that contains interfaces for a desktop computer, as well as circuits for analog to digital (A/D) and digital to analog (D/A) signal conversion, digital input/output devices (I/O), etc. To record EMG from the zygomaticus and corrugator sites, for example, one would need two EMG amplifiers that would connect to the base system. These systems typically contain customized acquisition and analysis software that is installed on a lab computer (see below). When evaluating potential systems, the EMG researcher should consider the selectable range of (a) the hardware filters, (b) amplification, and (c) sampling rate for A/D conversion. Specifically, the equipment should allow one to use a 500-Hz low-pass filter and a 10-Hz high-pass filter, 20,000x amplification, and the ability to sample the raw EMG signal at 1000 Hz (Fridlund & Cacioppo, 1986; Tassinary et al., 2007).

In addition to the equipment needed to record EMG signals, the startle eyeblink modification paradigm also requires the presentation of startle probes. Typically, these probes consist of a 105-dB white noise, with near instantaneous rise-time, and presented for 50 ms. The probes can be presented via headphones or speakers mounted near the participant's head. It is possible to create this white noise as an audio file that is presented on a computer with precise timing. The only additional equipment required is an audio amplifier and perhaps a dB meter to calibrate the intensity of the probe.

Specific computer requirements include having one computer dedicated to data acquisition and another dedicated to stimulus presentation, although the two tasks can be combined on the same computer for some applications. Most psychophysiological equipment comes with custom software for the acquisition of data during the experiment. Typically this appears on the experimenter's monitor as a scrolling window in which the data are plotted in real-time. The software may also contain various routines for quantifying the signal (e.g., integration or computing averages), or the researcher can use other commercially available software for more advanced analyses. Software for stimulus presentation usually does not come with the equipment, but this also means that the user is free to choose among several programs available for conducting experiments. The key requirement for any of these programs is the ability to send a digital output that marks events, such as when a trial begins or the participants makes a response. This digital output is sent to the acquisition computer so that events are time-locked to the EMG data that is collected.

Electrodes

When using bipolar recording (i.e., the voltage difference between two electrodes is amplified), two circular Ag/AgCl electrodes are needed for each recording site.

Fridlund and Cacioppo (1986) recommended that 0.50-cm diameter electrodes be used when recording from limb and trunk muscle areas, whereas miniature 0.25-cm diameter electrodes should be used for facial EMG recording. Note that manufacturers of electrodes offer a variety of sizes for the housing that surrounds the electrode; Fridlund and Cacioppo's recommendation refers to the actual detection surface of the electrode. In addition, because of the small size of EMG signals, the leads (i.e., wires) from the electrode to the pre-amplifier should be as short as possible – 1 meter should be adequate. One other consideration about electrodes is whether to use disposable electrodes, which come pre-gelled and are disposed after one use, or reusable electrodes, which can be used many times. In the long-term, using reusable electrodes is the more economical option, although they will begin to lose some of their conductivity with high use, and they do require vigilant cleaning and sanitization. Disposable electrodes work well, but they are usually used in conjunction with snap leads (i.e., the electrode snaps into a lead connector), which can be heavier, and therefore more noticeable, when attached to the participant's face.

Impedance meter

Another important component to an EMG laboratory is the impedance meter. Impedance, which is akin to resistance in a DC circuit, is typically not measured via commercially available multi-meters. Impedance meters are usually provided with EEG recording equipment, but they tend to be expensive because they are typically constructed to record the impedance from dozens of sites with a switch. Small, single-channel impedance meters are available from UFI Instruments, which provide a measure of impedance as either a digital reading (in kΩ) or, in the less expensive model, as a series of colored lights that indicate the extent to which the electrodes are at the recommended criterion of 10 kΩ.

Illustrative study

The illustrative study takes place in a two-room laboratory in which the participant enters the testing room without having to walk through the experimenter's control room. This set-up uses equipment from Biopac, but similar equipment is available via several manufactures (e.g., Contact Precision Instruments, ADInstruments, Mindware Technologies). Pre-amplifiers to record three channels of EMG (zygomaticus, corrugator, and orbicularis oculi) sit on a shelf behind the participant's head. An ethernet cable runs from the Biopac equipment through a hole in the wall to the control room where it connects to the computer that acquires the EMG digital signals. Also in the control room is an audio amplifier with headphones to present the startle probes to the participant. The audio amplifier is connected to a second computer in the control room that presents the

stimuli. An LCD monitor is mounted in front of the participant and the cable connecting it also runs through the wall to the control and is connected to the control computer. A small keypad sits on the participant's lap, and it is used to input responses when prompted to make a rating of a picture. A small camera is mounted directly in front of the participant, but is concealed by a screen. This camera is monitored in the control for participant movements, but is not connected to a video recorder.

In the control room sit two computers, a small television monitor that displays the video from the testing room, a sink, and storage space for electrodes, papers, etc. During the entire session, the experimenter continuously compares the display of EMG activity scrolling across the screen with the closed-circuit video from the participant's room. The software that comes with Biopac allows the user to insert comments into the record as they occur, thus allowing for remarks indicating that participant coughed, yawned, or asked a question, for example. An intercom system allows the experimenter to speak with the participant without having to enter the testing room.

Recording EMG and Blinks

Reducing participant anxiety

Participating in any psychophysiological experiment is often a novel experience for research participants. Moreover, the close proximity of the experimenter, who is cleaning the participant's skin and attaching electrodes, can heighten anxiety, and therefore greater muscle tension. And, as mentioned already, the testing session is a social situation itself, which can contribute to participant concerns about responding "appropriately." Tassinary et al. (2007) reviewed some of the following procedures to minimize participant anxiety:

1 *Before the experiment begins, have an introductory session and/or a tour of the laboratory facilities*. If time allows, give a video presentation for your participants, or provide a web-based virtual tour, in which you walk them through the steps they will go through when they arrive at the actual experiment. Show them exactly what the electrodes look like, what the experimenter will do to prepare the skin, what equipment will be used, etc.
2 *Use a less emotional word instead of "electrode" when speaking with the participant*. Because "electrode" easily connotes electricity and reminds the participant of more infamous studies, like the use of the supposed shock generator in Milgram's (1963) research, laboratory personnel should try to use "biosensors" or "sensors" instead. It is important to remember that all written documentation (e.g., informed consent forms) should also avoid the use of "electrode" whenever possible.
3 *Reduce the feeling of being watched*. As noted earlier, having the experimenter sit in a separate room and concealing the camera helps with this. In addition, avoid

making participants aware that they are being closely monitored by refraining from asking them about their movements (e.g., "did you just yawn?").

4 *Use a cover story and dummy electrodes.* Facial EMG researchers rarely explicitly tell their participants that the activity of their facial muscles is being measured. There are generally two reasons for this concealment. First, again, to reduce the feeling of being watched, using a cover story for the facial EMG electrodes, such as "we are recording activity that comes from the head, sort of like an EEG," is important. Second, if participants know that their facial muscles are being recorded, it may invoke social desirability problems (e.g., "I should probably being smiling now.") that may subsequently lead to exaggerated or even inhibited facial reactions that reduce the validity of the measure. The attachment of dummy electrodes (i.e., ones that don't actually record any activity) to the back of the neck and/or under the ears will also diminish the sense that the facial muscles are of interest.

Electrode preparation and attachment

Before the participant arrives at the experimental session, the electrodes can be prepared by attaching the adhesive collars to the electrodes. The collars that are commercially available usually require some trimming with scissors so that the two electrodes at each recording site are no more than 1 cm apart. The collars can be clipped at the points where they overlap. To reduce discomfort when later removing electrodes at the end of the experiment, trim the collars at any points where they may overlap with eyebrows and other facial hair.

After the participant arrives, high-conductivity gel can be inserted into the electrode wells, and they can be attached to the participant using the adhesive collars. Special attention should be given to minimizing the amount of gel applied to the skin (i.e., that which exceeds the electrode housing). Residual gel can create a conductive bridge on the skin area between the two electrodes, which will end up making recording the desired EMG signal impossible (Blumenthal et al., 2005).

To insure the lowest impedance possible, the skin must be cleaned and lightly abraded at each electrode site. Once the participant has removed any make-up and skin cream from the face, a gentle cleaning using cotton balls and hypoallergenic soap works best. Then, further rubbing with an abrasive skin pad (available from many psychophysiology suppliers) at the recording site will remove dead skin cells and some of the outer layer of the skin. It is important that all sites are dry and free of soap before attaching the electrodes.

Because facial muscle movements are typically symmetrical, researchers tend to put electrodes on one side of the face only. Specific guidelines for attaching electrodes to the zygomaticus major, corrugator supercilii, and orbicularis oculi regions are provided below. These are derived from Fridlund and Cacioppo (1986), as well as Tassinary, Cacioppo, and Geen (1989), which are excellent sources for recommendations of facial EMG placements (see Figure 3.2). Each is

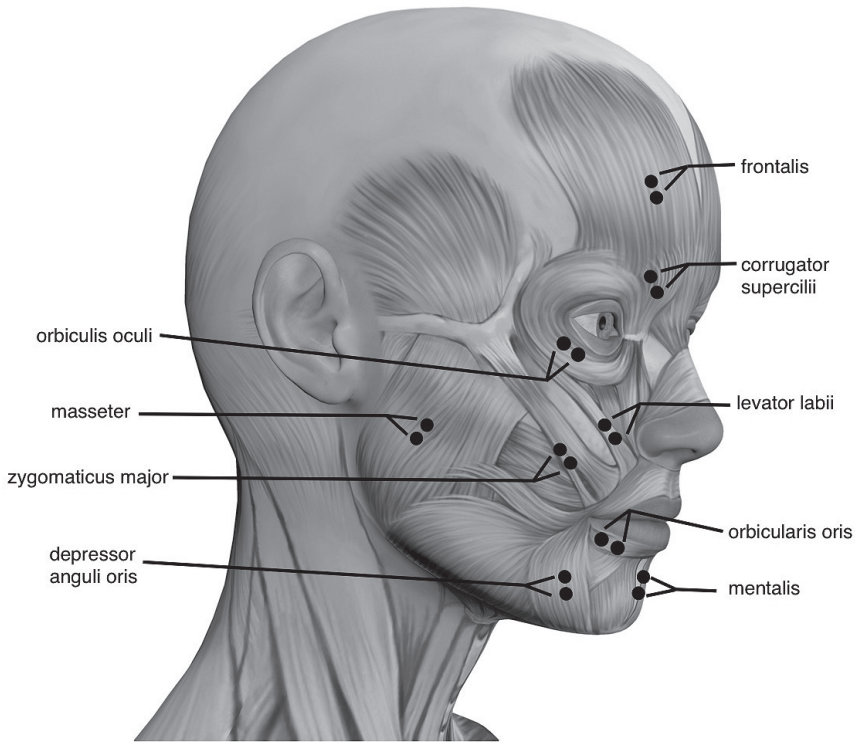


Figure 3.2 Common facial EMG placements and their corresponding muscles, based on Figure 12.4 in Cacioppo et al. (2007).

based on common facial landmarks such as the lip corner and inner canthus, as can be seen in the following:

- *Zygomaticus major* (cheek muscle): Draw an imaginary line from the corner of the lip (when at rest) to the preauricular pit (the small depression anterior to the helix of the ear). The first electrode should be attached 2.5 cm from the lip corner along this line. The second electrode should be attached 1 cm above the first electrode on this same line.
- *Corrugator supercilii* (brow muscle): The first electrode is attached directly above the inner canthus of the eye just above the eyebrow. The second electrode is attached lateral to the first electrode approximately equidistant from the eyebrow.
- *Orbicularis oculi* (eyeblink muscle): First electrode is placed below the bulge of the eye (or pupil when looking straight ahead) in the center near the edge of the lower eyelid. The second is placed 1 cm dorsal from the first approximately equidistant from the edge of the lower lid.

The recording of orbicularis oculi EMG can serve in the measurement of either Duchenne smiles (Ekman, 1992) or the startle eyeblink reflex. Although this is the most common site to record EMG for a startle response, the use of the postauricular reflex has also attracted recent interest (e.g., Benning, Patrick, & Lang, 2004; Hackley, Muñoz, Hebert, Valle-Inclan, & Vila, 2009; Hess, Sabourin, & Kleck, 2007), as it too seems to be sensitive to manipulations of emotion. It involves placing electrodes behind the ear to measure the auricularis posterior muscle, a vestigial muscle that is involved in moving the ear in other animals. Interestingly, although it does not appear to have any functional significance for modern humans, the postauricular startle reflex is enhanced when participants view pleasant stimuli and is attenuated when they view unpleasant stimuli (Benning et al., 2004). For more details, see Hess (2009).

Baseline/adaptation period

Once the electrodes are attached and recording of EMG activity has been verified, it is important that an adequate adaptation period be provided to allow the participant to get used to the experimental setting. In EMG studies, it is common to allow the participant to relax for approximately five minutes with eyes closed. Then, when the experiment actually starts, practice trials are presented to allow the participant to adapt to the procedures. These adaptation considerations are based on the goal of having the level of EMG activity as low as possible. It is common in psychophysiological research to compare physiological responses to baseline activity (e.g., increases in heart rate over the resting heart rate) because of the large variability in resting levels. With EMG measurement, in contrast, there is a "true" baseline in the sense that a muscle completely at rest will not be firing action potentials, and therefore the EMG activity should be zero. In a typical laboratory setting, however, muscles seldom show zero activity because the participant is not completely relaxed (Tassinary et al., 2007). Therefore, even with the inclusion of an adequate rest period and practice trials, a researcher may want to consider individual differences in basal EMG activity during data analysis.

One possibility is to record basal EMG during an extended resting period and then use average EMG activity at each site to compute a change score or to use as a covariate. The problem with this approach is that the use of one rest period at the beginning of the experiment will be temporally and substantively different from the experimental trials that occur later. The participant may become more relaxed by the end of the experiment, or the electrode connection could improve slightly (e.g., as the gel soaks into the skin, impedance levels can drop), which would mean that the basal EMG level would decrease over time. An alternative is to measure the EMG activity in the period just before each trial's onset that is comparable in length to the period of interest (e.g., Larsen & Norris, 2009; Schwartz et al., 1976; Winkielman & Cacioppo, 2001). For example, if the experiment involves multiple

trials consisting of 5-second presentations of the stimuli, activity during the 5-second epoch just before stimulus presentation could be considered the baseline. More discussion about the advantages and pitfalls of the use of prestimulus periods and baselines appears in Jennings and Gianaros (2007).

Alternatively, one could use a closed-loop baseline that involves requiring the participant to sustain EMG activity at some near-zero level before the onset of the next trial (McHugo & Lanzetta, 1983). This technique is advantageous compared to the use of simple change scores, which can be fraught with the violation of statistical assumptions and other procedural challenges. Closed-loop baselines, however, also have the potential of shaping participants due to idiosyncratic strategies and experimental conditions (Tassinary et al., 2007). For example, a participant may begin to realize that variation in the tension in their cheeks has an influence over how quickly trials are next presented. Tassinary et al. noted that it is this reason why most EMG researchers have not employed the closed-loop baseline procedure, although it is more routinely used in the investigations of muscle fatigue (e.g., Roy et al., 1997).

Checking EMG placements

Regardless of how well one follows the published recommendations for facial EMG placements, the possibility remains that, due to variation in the structure of the facial musculature, the target muscle could be located in a different location for a participant, or the muscle might be absent altogether. For example, 18% of cadavers were found to have a complete absence of the corrugator supercilii muscle, and 2% were missing the zygomaticus muscle (DuBrul, 1980). It would be ideal if the experimenter were to instruct the participant at the beginning of the testing session to move each of the target muscles to verify that they were being recorded, but this would likely draw attention to the face as a major point of interest. Alternatively, a convention developed by EMG researchers is to have the participant pose relevant facial expressions at the end of the experiment while EMG activity is recorded. For example, asking participants to smile or furrow their brows should generate a large EMG signal (greater than 100 μV) at the zygomaticus or corrugator sites, respectively. If a participant instead shows a small response or none at all, the data from this recording site could be eliminated from the analyses.

Startle eyeblink recording

In addition to the recommendations about the preparation and attachment of facial EMG electrodes, some issues are unique to the measurement of the startle eyeblink reflex. For example, cleaning of the skin over the orbicularis oculi should be gentler than at other recording sites, as it is easier to lower the skin's impedance at

this site and vigorous cleaning with an abrasive pad will be more uncomfortable for the participant. And, unlike other facial EMG recordings, baseline startle reactivity across participants is highly variable. For example, one study found that the blink magnitudes for the most responsive participants in a control condition were 30–45 times greater than the less responsive participants (Blumenthal, Elden, & Flaten, 2004). The recommended way to deal with this variability is to use percent (or proportion) change sores, which require the inclusion of "control" condition startles as a comparison (Blumenthal et al., 2005; Blumenthal et al., 2004; P. D. Jennings, Schell, Filion, & Dawson, 1996). If a research design calls for the presentation of several visual stimuli, for example, a few (3–4) startle-alone trials would be included in each block. The blink magnitude for the experimental trials in that block would then be computed as percentage change scores by subtracting the mean amplitude of the control startles in the block from the amplitude of that startle response in each experimental trial. More details about the analysis of startle data are provided below.

A review of procedures

For the trust illustration, when participants arrive at the laboratory, the experimenter tells them that the study is concerned about physiological processes involved in person perception. They are also informed that biosensors will be attached at various places on the head and neck to measure involuntary neural responses that emanate from the head, "sort of like an EEG."

Pairs of miniature Ag/AgCl electrodes are then be attached to the left zygomaticus and corrugator regions, following published guidelines (Fridlund & Cacioppo, 1986). To record the startle eyeblink as EMG activity, another pair of electrodes is attached directly over the region of the left orbicularis oculi inferior muscle, the first electrode just below the pupil and the second 1 cm lateral to the first. A ground electrode is also attached to the left earlobe.

Participants then sit in a comfortable reclining chair wearing headphones for the remainder of the experiment. After the electrodes are attached, a 5-minute resting period tales place, at the end of which three presentations of the startling white noise burst are presented as examples. The participants then view the 36 photos of male faces: one half that were previously rated as trustworthy and the other half as untrustworthy faces. Each photo is presented for 5 seconds. Intertrial intervals will vary between 25 and 35 seconds.

The 36 trials are to be organized into three blocks of 12 trials. Six of these 12 trials consist of photos of trustworthy faces and six consist of untrustworthy faces. The startle probe is presented on four of the six trials that comprised each type of face, whereas the remaining two are considered "clear" trials, during which zygomaticus and corrugator EMG activity will be later analyzed. Each of the startle probe trials contains a probe at one of two lead intervals (300 or

4500 ms following slide onset). The order of the events across the 12 trials within each block is randomly determined and counterbalanced across participants, with the restriction that the first trial of the first block must always be a clear trial.

The startle probe is presented binaurally through Radio Shack Nova 28 headphones. It is generated by a Grason-Stadler noise generator and controlled by a Coulbourn audio mixer/amplifier to produce a 105-dB (A) SPL white noise burst, 40 ms in duration, with a near instantaneous rise/fall time. Intensity is calibrated on a Realistic Sound Level Meter using a Grason-Stadler earphone coupler appropriate for the headphones.

In addition to the probes presented during the slide presentations, startle stimuli are also presented during 18 of the intertrial intervals, 6 occurring during each trial block. These probes are presented at random intervals between 10 and 20 seconds into the intertrial interval. Responses to these probes serve as baseline measures with which to compare blink amplitudes elicited during the pictures. EMG signals are full-wave rectified and smoothed using a contour-following integrator with a time constant of 0.05 s. Activity will be digitized at 1000 Hz. All digitized signals are stored on a hard disk for later scoring.

For the startle probe trials, the amplitudes of all eyeblinks are computer scored offline based on published criteria. The amplitude of each eyeblink elicited during a slide presentation is expressed as a percent change score from the mean of the amplitudes of the blinks elicited during the intertrial intervals in each block. A positive modification score indicates that the blink during a slide was larger than the average blink elicited during the intertrial intervals, whereas a negative blink modification score indicates that the blink during a slide was smaller than the intertrial blinks. Trials in which no blink occurred in response to the probe are scored zero. In addition, blinks are discarded if the data are too noisy to be scored by the computer or the experimenter notes excessive movement at the time the startle probe was presented. For the clear trials, mean EMG amplitudes will be computed for each trial in which no artifacts are detected.

Data Reduction, Analysis, and Inference

EMG data files

As noted earlier, EMG data files typically consist of digitized samples of raw EMG recorded continuously during an experiment. Event marker channels indicate when specific trials began, a startle stimulus is presented, and/or, depending on the program and equipment available, the trial type. With three EMG channels recorded at 1000 Hz for 40 minutes, the binary file that is created during acquisition is relatively small – perhaps just a few hundred megabytes. Thus, the storage

and transmittal of EMG files are easy in the modern computing environment. In the sections that follow, some common data reduction strategies for discrete facial EMG and startle eyeblink modification are reviewed separately. It is assumed that the experimenter has one data file for each participant's testing session that can be analyzed with specialized data reduction software.

Discrete facial EMG recordings

The raw EMG digitized at an individual channel is a pseudorandom AC signal, which means that it has both positive and negative waveforms, and its average will therefore approximate zero. For this reason, EMG researchers base their analyses on the rectified and integrated (smoothed) EMG signal (Hess, 2009); both can be done offline with most data reduction programs. Rectification essentially means that all negative-going waveforms are flipped so that they become positive. Integration, which is a moment-by-moment estimate of the energy of the EMG signal over some unit of time (usually 5 ms), is widely used because it more closely related to actual muscle contraction than other smoothing techniques (Basmajian & De Luca, 1985; Lippold, 1967; Tassinary et al., 2007). Traditionally, integration was done online using a contour-following integrator, which provided an electrical output that reflected a running-average of ongoing EMG activity, based on a varying voltage potential proportional to the envelope of the signal (Tassinary et al., 2007). As noted, however, recent advances in personal computers make digital integration so feasible that it is actually difficult these days to find a manufacturer of psychophysiological equipment that includes contour-following integrators as part of the EMG equipment.

Of course, in addition to rectification and integration, current software packages also offer a variety of digital filters that can be used in conjunction with, or instead of, the hardware filters that are built into the EMG amplifiers. For example, the potential effects of any 50/60 Hz electrical noise in the participant's recording room can be directly examined by analyzing the data with and without a 50/60 Hz notch software filter. Similarly, one can adjust low- and high-pass filter settings more easily with software, as the researcher can always return to the original raw signal if a concern is raised about what filter settings should be used. By contrast, if one uses a more limited bandwidth with online, hardware-based filters during data acquisition, it is impossible to widen this bandwidth (e.g., lowering frequency of the high-pass filter) during later offline data analysis.

A final and optional step in the reduction of facial EMG data is to remove activity that reflects known artifacts, such as spontaneous blinks, yawns, or other overt activity. Such artifact editing requires either (a) a full video record of the testing session in which the artifacts can be time-locked to the EMG data

file (e.g., a yawn that appears 4 seconds after the second trial begins can be easily identified in both the video and the EMG file), or (b) diligent record keeping by the experimenter who observes the participant's face throughout the experiment. Removing the artifact itself can involve replacing the offending waveform with an average of the remaining activity, or simply shortening the interval in which the EMG is averaged (see the next section). Although artifact editing can reduce noise (and error variance), it involves the deletion of EMG activity that may be relevant to the phenomenon of interest. Thus, it is recommended that such editing be done only after consideration of whether the artifacts may simply be dealt with as random noise that varies across all conditions and participants.

Computing Mean EMG Activity

Once the EMG signal has been rectified, integrated, filtered, and edited, the next step is to compute the mean activity over the specified time period. For example, the mean amplitude for a 5-second interval sampled at 1000 Hz would be the mean (positive) voltage across all 5000 data points recorded for one channel. The output file created by this process typically consists of one mean value for each channel recorded during the entire trial epoch. It is also fairly easy to compute means for smaller intervals, such as 1-second periods. In this case, one could take the mean of the shorter epoch means at some later point, but still be able to examine the time course of a response with more precision. For example, a stimulus might elicit a response only after a participant has had a few seconds to process it. If the mean were computed over a 10-second epoch following stimulus onset, averaging could lead to an underestimate of the treatment's effect because the seconds in which there was no EMG activity would be averaged in with the few seconds in which there was a burst of EMG activity. By contrast, a second-by-second analysis of means could reveal the EMG burst appearing only after a set amount of time. Moreover, if a researcher is concerned about variability in baseline EMG, the mean activity of a pre-stimulus period, which should be equal to the length of trial epochs, can also be computed and used to derive a change score for each trial. Larsen and Norris (2009), for example, computed mean activity for each second of a 6-second picture viewing trial, and then subtracted the activity from the 1-second period immediately preceding stimulus onset, thus creating six change scores for each experimental trial at each muscle site. They then compared the change in EMG activity at the zygomaticus major region on a second-by-second basis when participants viewed moderately pleasant pictures presented in the context of mildly pleasant contextual pictures to when they viewed such pictures presented in the context of extremely pleasant pictures (see Figure 3.3).

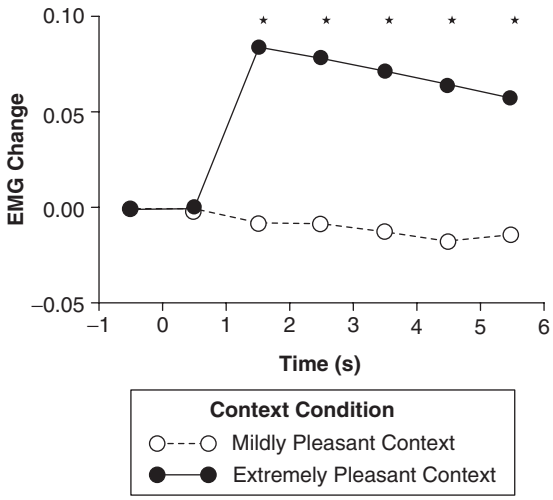


Figure 3.3 An example of using EMG change scores, computed by subtracting the mean EMG activity in the 1-sec period preceding stimulus onset from the each of six 1-sec periods during picture viewing. Larsen, J. T., & Norris, J. I. (2009). A facial electromyographic investigation of affective contrast. *Psychophysiology, 46*, 831-842. Reprinted with permission.

Computing other EMG parameters

Although most social psychological research using EMG has used the mean amplitude of the signal as the dependent variable, simple averaging may lead the researcher to miss other characteristics of the signal that possibly have psychological relevance. In fact, simple averaging of the integrated signal is probably more appropriate when the underlying muscle movements are forceful, extended in duration, and overt (Tassinary et al., 2007). This is because EMG signals that consist of low rates of firing (small numbers of motor units are recruited) tend to generate poorly fused and noisy integration. The irony here, of course, is that the application of EMG to measure facial activity in a social psychological context involves muscle movements that are usually weak, brief (1–2 second bursts are common), and covert (often the participant never makes a noticeable facial movement during the entire experimental session).

One alternative to computing mean amplitudes of the integrated signal is to analyze the topography of the EMG signal itself, which means that temporal information is considered in addition to the amplitude domain (Cacioppo, Marshall-Goodell, & Dorfman, 1983; Hess, Kappas, McHugo, Kleck, & Lanzetta, 1989). This could involve the use of a waveform moment analysis, which is a method that summarizes a non-rhythmic waveform, such as the

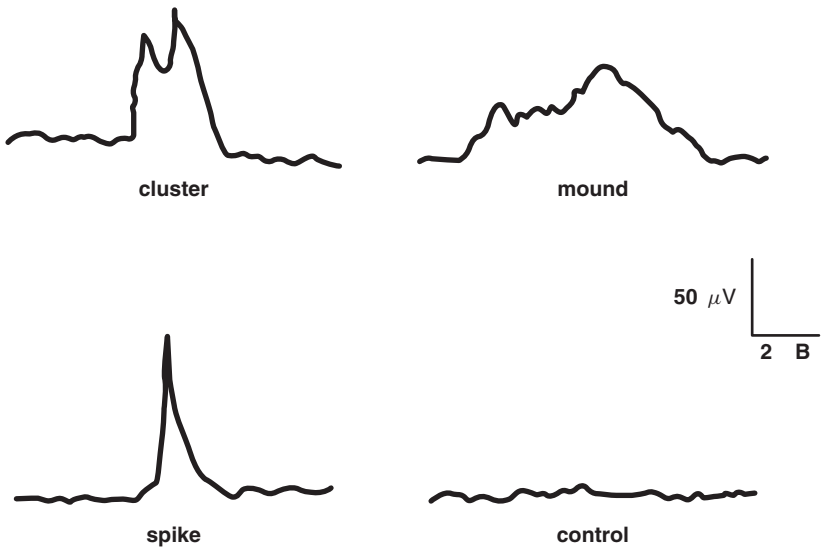


Figure 3.4 Four possible waveforms used by Cacioppo et al. (1988) to test whether participants had fleeting thoughts or emotions that they did not reveal during a clinical interview. Cacioppo, J. T., Martzke, J. S., Petty, R. E., & Tassinary, L. G. (1988). Specific forms of facial EMG response index emotions during an interview: From Darwin to the continuous flow hypothesis of affect-laden information processing. *Journal of Personality and Social Psychology, 54*, 592–604. Adapted with permission.

EMG signal, with a set of indicants derived from the mathematical moments of the waveform (see Dorfman & Cacioppo, 1990, for more details). Another way to examine the topography of the signal is to look for specific waveforms that possibly index the psychological operation of interest. For example, Cacioppo et al. (1988) identified four specific forms of the corrugator EMG response that could occur while a participant self-disclosed during an interview. As seen in Figure 3.4, these were identified as a spike (a sudden, short burst of EMG activity), a cluster (two or more partially overlapping groups of EMG spikes), a mound (a relatively smooth response with a gradual onset and offset), and a control waveform (baseline activity with no noticeable EMG bursts). Once the participant had completed the interview, the researchers identified 10-second segments in which one of the four waveforms occurred. The participants then watched short playbacks of their interview that corresponded to these segments and were asked about any feelings or thoughts that they may have not disclosed originally. Clusters were found to be associated with stronger negative emotions and weaker positive emotions than were spikes and mounds. Simple averaging of the EMG signal would presumably

have obfuscated the relationship between a specific corrugator EMG response and a fleeting thought or memory that was not originally expressed. The findings from this study highlight the potential benefit of analyzing EMG data as function of time and amplitude, but, surprisingly, few, if any, facial EMG studies since have reported using this type of analysis.

Startle EMG

The startle eyeblink is a burst of EMG activity with a brief duration. It is quantified by identifying the peak amplitude of the EMG waveform in a specified time window; typically 20 to 150 ms after the onset of an acoustic startle noise (Blumenthal et al., 2005). The maximum value of EMG activity during the window is considered the peak, and the oculi EMG activity just prior (e.g., 25 ms) to the onset of the startle stimulus is considered the baseline. Thus, one computes the amplitude of a startle eyeblink response by subtracting the baseline activity from the peak. In the case where no blink occurs in response to the stimulus, the amplitude is zero. If needed, the response latency can be determined by searching through the EMG waveform after the startle onset for the point in which there is a significant increase above the baseline EMG. What constitutes a "significant increase" could be the first point that is three times greater than the average of the baseline (e.g., Grillon & Davis, 1995) or where the slope of the EMG signal exceeds some criterion (Blumenthal et al., 2005; Brinkworth & Turker, 2003).

Like facial EMG quantification, the scoring of EMG startle responses can be done manually or automatically with computer-assistance, and several programs are available to the researcher. When using a manual method the scorer can select, on a trial-by-trial basis, the peak for each blink, and the software can then calculate the amplitude and latency of the response. An advantage of this method is that visual inspection allows a researcher to accept or reject questionable responses. In a fully automated method, the software scores all trials at once based on user-defined parameters. Although this saves time during the data quantification stage, it can also mean that erroneous data will be included if spontaneous blinks or other artifacts (e.g., squinting) happen to occur during the scoring window of a trial.

Once the amplitudes (and onset latencies) from all trials have been determined, it is common to again average these amplitudes across all trials in a condition and then perform inferential statistics on those condition means. When averaging across blink responses, however, a distinction is made between blink magnitude and amplitude. Blink magnitude is used when the condition average includes values of zero for trials where there is no startle response. Blink amplitude is used when the condition average was calculated without non-response trials. Because researchers are more likely to include trials with no responses in the

computation of condition averages, blink magnitude is the more commonly used term (Blumenthal et al., 2005).

Inferential issues

J.R. Jennings and Gianaros (2007) provided a comprehensive review of the statistical issues relevant to psychophysiological data, and therefore much of what appears there applies to the analysis of EMG and startle eyeblink modification data. More specifically, these methods warrant some unique consideration during data analysis. For instance, EMG amplitudes are often positively skewed, so it may be appropriate to submit the data to a square-root transformation prior to analysis (e.g., Larsen & Norris, 2009). For startle eyeblink data, wide individual differences in blink magnitude are often obtained. Thus, extreme responses (i.e., unusually large blinks) can have a disproportionate influence on the inferential analyses. As mentioned earlier, one solution is to use percent change scores, based on subtracting the baseline blink response from the trial blink (Blumenthal et al., 2004). Alternatively, one can eliminate outlier participants or trials (e.g., more than 3 SDs from the mean, Blumenthal et al., 2005). A similar issue has been raised with variability of facial EMG. Bush, Hess, and Wolford (1993) found that using within-subjects z-transformations in a Monte Carlo study increased power, as it reduced variance by standardizing data based on each participant's response. This transformation, however, is not appropriate when between-subjects factors are part of the analysis, as may be the case when comparing participants from two different groups. Fridlund and Cacioppo (1986) recommended caution when using such transformations with EMG data, and their use should always be explicitly justified. The most prudent approach is to compare and contrast the effects of both transforming the data and leaving it untransformed (Hess, 2009).

Hypothetical study of trust: analysis

For the illustrative study of trust, the data are inspected for outliers and normality. Based on this data screening, assume for this example that there is no need to conduct further data transformations. The EMG data collected during the clear trials are measured as change scores representing the difference between the activity over the entire 5-second presentation of the face and the 5-second period preceding the trial. Any change score above or below 3 SD from the grand mean is removed and the remaining trials of that trial type will still be used for analysis. For the startle data the primary analysis is conducted as an ANOVA using a multivariate approach, comparing participants' mean percent change startle for the two target faces (trustworthy versus untrustworthy) at each lead interval (300 or 4500 ms). The EMG data during the clear trials can

be analyzed as t-tests comparing trustworthy to untrustworthy faces to both the zygomaticus and corrugator change scores.

Conclusion

This chapter has reviewed the why's and how's of electromyography, with an emphasis on facial EMG and startle eyeblink modification as measures of affective and cognitive processes. Swift advances in computers and psychophysiological equipments in the past two decades have made these methodologies relatively inexpensive and easy to use, yet they remain powerful tools for social psychology and related fields. In the early 1980s, it was presumed that a researcher must have a great deal of expert guidance and training to set up and conduct studies using EMG. Since then, however, manufacturers of psychophysiological equipment have made their products more user-friendly, and the software programs available for stimulus presentation, acquisition, and data analysis no longer require the special skills in programming needed 25 years ago. Hopefully, this chapter has convinced anyone interested in these methods to include them in their own investigations.

As one final example of the promise of EMG to increase our understanding of social behavior, consider a recent study that used EMG to test hypotheses derived from recent work on the human mirror neuron system, which appears to map the actions performed by others onto a motor representation of those same actions in the observer (Fabbri-Destro & Rizzolatti, 2008). In Cattaneo et al. (2007), two groups of participants, high-functioning children with autism and typically developing children, observed an experimenter perform two actions – grasping food to eat or grasping a piece of paper to put into a container. Each action began with the same arm movement of reaching for the object, and each took less than 4 seconds to complete. While the children watched these actions, surface EMG was recorded from the mylohyoid muscle region on the neck, a muscle that widens the oral cavity in preparation for eating. As can be seen in Figure 3.5, the typically-developing children showed an increase of mylohyoid EMG activity whenever the experimenter began to reach for food, but not when reaching for a piece of paper. That is, those participants appeared to anticipate the muscle activity that is required for eating (just as the experimenter's own mylohyoid would have increased prior to eating) via the mirror neuron system. In contrast, the children with autism didn't show this anticipatory mirroring activity, even though they fully understood the experimenter's actions and could perform them themselves. The authors concluded from these findings that damage to the motor system, which appears to be essential in action and intention understanding, may play an important role in the deficits that are observed in autism. This study merits attention here because the researchers used EMG in a rather novel way to study a fundamental aspect

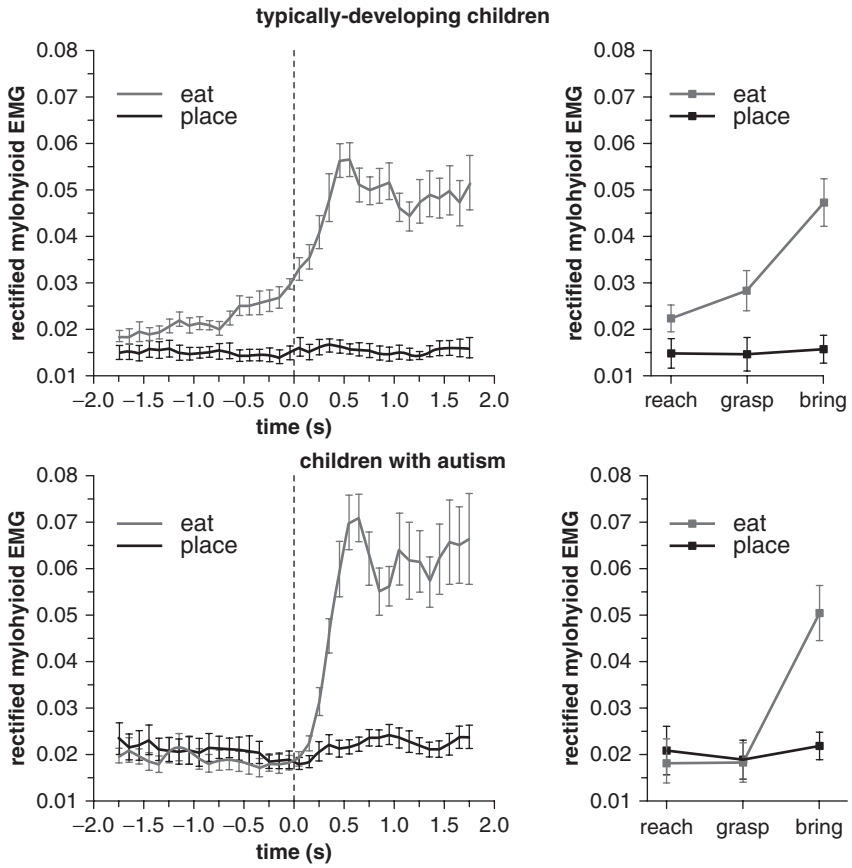


Figure 3.5. EMG activity recorded from the mylohyoid muscle region while typically-developing children and children with autism watched an experimenter grasp a piece of food to eat or a piece of paper to place in an container. From: Cattaneo, L., Fabbri-Destro, M., Boria, S., Pieraccini, C., Monti, A., Cossu, G., & Rizzolatti, G. (2007). Impairment of actions chains in autism and its possible role in intention understanding. *Proceedings of the National Academy of Sciences, 104*(45), 17825–17830. Copyright 2007, National Academy of Sciences, USA.

of social interactions. More interesting research using EMG and startle reflex modification in either "traditional" or novel ways is certainly forthcoming, and it will continue to yield more advances in social psychology, cognitive neuroscience, and social neuroscience.

4

Endocrine Measures: Cortisol

Interest in the hormone cortisol, the end product of activation of the hypothalamic-pituitary-adrenocortical (HPA) axis, has been growing among social psychologists. Studies that assess this hormone have been increasing nearly exponentially in the literature. Research has linked a number of social psychological processes to patterns of HPA activity, which have implications for our understanding of intergroup relations (e.g., Amodio, 2009; Mendes, Gray, Mendoza-Denton, Major, & Epel, 2007), interpersonal processes and close relationships (e.g., Kiecolt-Glaser et al., 1997; Stroud, Tanofsky-Kraff, Wilfley, & Salovey, 2000), emotion (e.g., Dickerson, Mycek, & Zaldivar, 2008; Lerner, Dahl, Hariri, & Taylor, 2007), self-regulation (e.g., Creswell, Welch, Taylor, Sherman, Gruenewald, & Mann, 2005; Dandeneau, Baldwin, Baccus, Sakellaropoulo & Pruessner, 2007), and cultural differences (Taylor, Welch, Kim & Sherman, 2007). Identifying the physiological correlates of social psychological processes also allows an extrapolation to situations or contexts that, if repeatedly encountered, may lead to downstream health consequences, as persistent activation of the HPA system has been linked with negative health outcomes (e.g., McEwen, 2004). For these reasons, many have been encouraged to begin incorporating endocrine biomarkers into their research.

However, there are a number of theoretical and methodological factors that must be considered when examining neuroendocrine hormones, such as cortisol, in the context of social psychological research. For example, decisions including the time of day in which the study is run and timing of cortisol assessment can be critical for capturing a cortisol response in the laboratory. Task selection can also be essential; not all stressors trigger the HPA system, and so certain stressors with specific characteristics need to be used in order to effectively elicit a cortisol response. Additionally, a number of health behaviors and health conditions can influence HPA reactivity, and so exclusion criteria and restrictions prior to the experimental session should be instituted in order to maximize the validity and interpretability of the cortisol data. This chapter focuses on reviewing these conceptual, methodological, and procedural factors (see Table 4.1).

Table 4.1 Factors to consider when conducting cortisol research in the laboratory

Methodological Factors
Time of Day
Timing of Assessment
Number of Assessments
Cortisol Sampling Method (blood, saliva)
Stressor/Task Characteristics
Task Category
Uncontrollability
Social Evaluation
Novelty
Stressor Length
Individual Differences/Health Behaviors of Participants
Gender
Hormonal Status (menstrual cycle phase, oral contraceptives, menopausal status)
Pregnancy
Age
Smoking
Exercise/Obesity/Diet
Sleep
Physical and Psychological
Health Conditions
Medications

Biological Background Information

Activation of the HPA axis begins when the hypothalamus releases corticotrophin releasing hormone (CRH) that in turn stimulates the anterior pituitary to secrete adrenocorticotrophin hormone (ACTH), which then leads to the release of glucocorticoids (cortisol in humans and corticosterone in non-human animals) from the adrenal cortex. In more detail, neural signals trigger the paraventricular nucleus (PVN) of the hypothalamus to produce CRH. This neuropeptide is transported via the portal vein to the anterior pituitary, where it stimulates the production of the protein proopiomelancortin, which is cleaved into ACTH and beta-endorphin. The ACTH is then released in a pulsatile fashion into the bloodstream that, in turn, causes the adrenal cortex to increase the production and secretion of cortisol. When cortisol is released systemically, it exerts a number of effects on cells and systems of the body.

The HPA axis is regulated by a negative feedback process. When cortisol levels become elevated, the production and secretion of the HPA axis hormones is suppressed both at the level of the hypothalamus and anterior pituitary (resulting in less CRH and ACTH in circulation). Additionally, the hippocampus is involved in this feedback loop. When cortisol binds to glucocorticoid receptors on the hippocampus, it serves as a "brake" that also inhibits HPA activity.

Thus, the hippocampus is able to regulate the HPA axis and suppress the release of its hormones.

The HPA axis is important for supporting normal physiological functioning and regulating other physiological systems. Cortisol is particularly involved in metabolic functions; it releases energy stores (primarily by stimulating the conversion of amino acids to glucose, and breaking down fat and protein stores in the body), which elevates blood glucose levels and provides fuel for the body. Cortisol can also regulate other physiological systems, such as the immune system. For example, cortisol is an anti-inflammatory agent that can suppress certain aspects of immune functioning (e.g., proinflammatory cytokine production). Cortisol also has permissive effects, which allow other systems to operate effectively. For example, certain levels of cortisol are necessary for the hormones of the sympathetic nervous system (e.g., catecholamines) to exert effects. Cortisol is critical for maintaining essential metabolic and physiological functioning, as well as playing an important role in organizing the body's response to stressors including threat.

In the presence of a stressor or threat, the HPA axis receives inputs from brain regions associated with the integration and appraisal of environmental stimuli (e.g., thalamus, prefrontal cortex) and the generation of emotional responses (e.g., amygdala, hippocampus). This can lead to activation of the HPA axis, and subsequent release of CRH, ACTH, and cortisol. The short-term activation of this system in the context of a stressor is thought to be adaptive, by releasing energy and regulating other physiological systems to deal with the acute demands. However, if the system is turned on too often or for too long, it is thought to have a number of negative effects on physiological processes and health outcomes. For example, it can lead to suppression of the immune system, damage to hippocampal neurons, and the development and exacerbation of a number of different diseases (e.g., depression, cardiovascular disease, diabetes; for review, see McEwen, 1998, 2004).

General Issues for Cortisol Research

Types of studies that assess the HPA axis

There are different ways to conceptualize and assess HPA activity. These methodologies can be grouped into three primary categories: those that examine patterns of HPA activity across the day (diurnal rhythm), those that examine the HPA response to awakening (cortisol awakening response, or CAR), and those that examine HPA responses to stressors in the laboratory or in naturally-occurring contexts. The first two types are briefly reviewed below, before focusing on laboratory stress reactivity methodology and procedures.

Assessing diurnal rhythm and cortisol awakening response

Cortisol has a diurnal pattern in which levels vary dramatically throughout the day. Rather than being secreted continuously over 24-hours, cortisol is released in pulses that correspond to the frequency and magnitude of ACTH surges from the pituitary (Veldhuis, Iranmanesh, Johnson, & Lizarralde, 1990). Pulse amplitude is greatest in the morning; cortisol levels increase dramatically upon awakening, and then show a pronounced decrease throughout the late morning. Levels tend to stabilize and flatten throughout the afternoon and early evening, reaching the lowest nadir in the late evening/early morning hours.

While this is the typical cortisol trajectory over the course of the day, there are a number of factors that can lead to perturbations in the diurnal rhythm. For example, this circadian profile is associated with the sleep–wake cycle (Spath-Schwalbe, Uthgenannt, Voget, Kern, Born, & Fehm, 1993), and can be disrupted with changes in sleep patterns and sleep interruptions. Events including physical activity, meals, and stressors can be superimposed on this diurnal rhythm, leading to fluctuations in circadian changes throughout the day. Additionally, there are many individual difference factors that have been associated with alterations in the diurnal rhythm, including physical disease states (e.g., cancer; Sephton, Sapolsky, Kraemer, & Spiegel, 2000), psychological disorders (e.g., depression; Heim & Nemeroff, 1999), and individual differences (e.g., age, gender, socioeconomic status; Cohen, Schwartz, Epel, Kirschbaum, Sidney, & Seeman, 2006; Stone et al., 2001; Van Cauter, Leproult, & Kupfer, 1996). Identifying the factors associated with alterations in diurnal patterns has been a fruitful line of inquiry, and an important avenue for research on HPA activity (for guidelines, see: www.macses.ucsf.edu/Research/Allostatic/notebook/salivarycort.html#Samples).

Conceptually related, but distinct, from this diurnal cortisol pattern is the cortisol awakening response (CAR). Within approximately 30 minutes after awakening, there is a substantial increase in cortisol levels of 50–75% (for review, see Fries, Dettenborn, & Kirschbaum, 2009), which is an indicator of HPA functioning. The CAR is relatively stable over time and is detectable and observed in the majority of participants. Individual differences in demographic, health, and sleep factors have predicted the magnitude and trajectory of this response (e.g., Clow, Thorn, Evans & Hucklebridge, 2004; Fries et al., 2009). Additionally, the CAR is sensitive to stress-related processes, with an increased CAR observed among those experiencing chronic stressors or overload (e.g., Steptoe, Cropley, Griffith, & Kirschbaum, 2000; Wust, Federenko, Hellhammer, & Kirschbaum, 2000) and those anticipating an upcoming stressor (e.g., Rohleder, Beulen, Chen, Wolf, & Kirschbaum, 2007).

Assessing HPA responses to stressors

Studies that have mapped diurnal rhythms and/or the cortisol awakening response provide a window into certain aspects of HPA functioning, and have led to important

insights into how stressors, social processes, and individual differences can modulate HPA activity. Other studies have focused on how the HPA axis responds to stressors, either in the laboratory or in naturally-occurring settings. This methodology allows researchers to chart how certain stressors can activate physiological responses, and how social and personality processes may be associated with these effects.

There has also been a great deal of interest in stress reactivity studies, as patterns of HPA reactivity could have implications for health. Experiencing frequent or chronic stressors, coupled with prolonged or sustained cortisol activation, could result in over-exposure to HPA hormones. This could occur via exaggerated *reactivity* (peak increases in cortisol levels), or failure to *recover* (return to baseline after the stressor ends). The focus of the majority of research in this area has been on reactivity, or the magnitude of peak change from baseline; however, recovery may also be important, as failing to shut down this HPA system after a stressor ends could lead to greater exposure to these hormones, which could have implications for disease (e.g., Linden, Earle, Gerin, & Christenfeld, 1997; McEwen, 1998). Additionally, hypo-reactivity, or failing to mount a cortisol response when warranted, has received increasing attention in the literature, and has also been linked with negative health outcomes (Heim, Ehlert, & Hellhammer, 2000). Understanding the specific types of stressors or individual differences that predict heightened reactivity, failure to recover, or hyporeactivity could be important; this could delineate certain situations which, if experienced chronically, may be more likely to lead to negative health outcomes, or identify individuals who may be particularly vulnerable in certain stressor contexts.

Researchers have examined HPA responses to stressors both within and outside of the laboratory. Laboratory research allows for standardization of the stressor task and control over the experimental context and confounding variables; furthermore, it allows for causal inferences between stressor conditions and HPA responses. However, there are questions regarding whether laboratory research is generalizable; in other words, do patterns of HPA reactivity in the lab mirror patterns of reactivity in daily life? This balance between standardization and control on the one hand and generalizability on the other is important to consider when designing protocols that assess HPA responses. Research programs that move back and forth between the lab and "real life" may maximize the benefits of both types of investigations; for example, testing responses to a social-evaluative performance stressor in the lab (e.g., speech/math task in front of an evaluative audience; Kirschbaum, Pirke, & Hellhammer, 1993) and responses to a social-evaluative performance stressor in real life (e.g., judged ballroom dancing competition; Rohleder et al., 2007). Additionally, some have examined "real-life" social processes in the lab; for example, having couples discuss a problem in their marriage (e.g., Kiecolt-Glaser et al., 1997) or experience social rejection (e.g., Stroud et al., 2000), which may more closely mirror naturally occurring situations. The degree of generalizabilty and applicability of laboratory

stressors to real-life situations is important to consider when selecting a stressor task in laboratory research.

Methodological Considerations for Cortisol Research

A number of methodological characteristics can influence whether a cortisol response is: a) elicited, and b) captured in the laboratory. Some of these factors are procedural, including the time of day in which the study is conducted, and when and how cortisol is assessed. Others include components associated with the stressor itself (e.g., type of task, characteristics of the task, etc.); these are important, as not all laboratory stimuli are capable of eliciting cortisol reactivity. The methodological factors that can impact the ability to both trigger and capture cortisol responses in the laboratory are reviewed below.

Diurnal rhythm

The diurnal rhythm of cortisol and the cortisol awakening response are two time-of-day dependent phenomena that should be taken into account when designing and interpreting cortisol reactivity studies. The diurnal rhythm of cortisol has critical implications for determining optimal times for running participants through cortisol reactivity investigations. Because cortisol levels vary widely throughout the day, it is important to make sure that time is kept constant as much as possible across participants; baseline values among participants run in the morning would be very different from those run later in the day. Controlling for diurnal rhythms by running all participants at the same time of the day could lead to greater interpretability and comparability of the results.

Because of the relative stability of cortisol levels in the afternoon hours (compared to decreasing levels in the morning), it could be easier to observe increases in this parameter during this time of the day. Indeed, a meta-analytic review of 208 acute psychological stressor studies found that time of day was a significant predictor of cortisol reactivity (Dickerson & Kemeny, 2004). Studies conducted during the afternoon were associated with an effect size of $d = 0.46$, while those conducted in the morning had an effect size of $d = 0.14$, demonstrating that cortisol reactivity is nearly three times greater in the afternoon compared to the morning. This suggests that it is optimal to run all participants in the afternoon so as to not miss important stress-related changes in this system.

While afternoon may be ideal, it is not the case that stress-induced increases in HPA activity can only be observed in the morning; in fact, many studies have shown that this is possible. For example, Kudielka and colleagues (Kudielka, Schommer, Hellhammer, & Kirschaum, 2004) compared participants who performed the Trier Social Stress Test (TSST; Kirschbaum et al., 1993), a standardized

stressor in which speech and mental arithmetic tasks are performed in front of an evaluative audience, at different times of the day. They found no differences in reactivity on the part of participants run in the morning versus afternoon across different indicators of HPA activity (ACTH, plasma cortisol, salivary cortisol), suggesting that absolute increases in HPA activity did not differ by time of day. However, they did observe that basal levels of HPA parameters were higher in the morning; and further, elevated baseline levels predicted reduced reactivity. Therefore, the degree that cortisol levels are elevated in the morning may correspond to reductions in cortisol reactivity at that time.

If studies are conducted in the morning, including a no-stressor control group or a within-subjects design to chart naturally declining cortisol levels could be important for making appropriate conclusions about cortisol reactivity. This is important, as during the morning hours a stress-induced "increase" in cortisol levels could actually manifest as no absolute change; having a comparison of naturally-declining values under resting conditions would allow one to test if cortisol declines *less* following an acute stressor than would be typical for that time of the day. Additionally, if participants are run in the morning it may be more important to select a stressor that is a strong elicitor of cortisol reactivity (e.g., TSST; see below) that could potentially overcome the morning declines. Conversely, it may be more important to conduct studies in the afternoon when utilizing tasks that mildly or moderately activate this system, in order to increase the likelihood of observing and capturing potential stress-related increases in this system.

Finally, if participants are run in the morning, it is also important to make sure that the cortisol awakening response (CAR) is missed in data collection (e.g., participants run at least 1 hour post-awakening). Rapid increases in cortisol are seen during this window, and stress-induced perturbations could be masked during this time.

In summary, there are a variety of time-of-day issues to consider when conducting laboratory cortisol reactivity research. It is important, at the very least, to run all participants at the same time of the day, as it would be difficult to compare across participants and draw meaningful conclusions if this is not done. It may be beneficial to test in the afternoon, to maximize the chances of observing stress-induced changes, particularly if employing a mild psychosocial stimulus.

Timing

The timing of assessments of HPA activity can have a major impact on the investigator's ability to quantify and capture a response in the laboratory. Because it takes time to activate the HPA axis following psychosocial stimulation, stress-induced elevations in cortisol are not immediately observed. Therefore, the proper timing of sample collection is critical for drawing appropriate conclusions about patterns of HPA activity.

Baseline assessment Adequate assessment of baseline cortisol levels is important for interpreting results of reactivity studies. A pre-stressor sample is necessary to determine whether a cortisol response has been elicited, and provides an anchor for comparing post-stressor values. Even in studies in which the focus is on post-task differences between experimental conditions (e.g., comparing post-stressor cortisol levels when performing a speech in the presence or absence of a social-evaluative audience), the baseline assessment is vital for understanding the meaning of the results. Without a baseline assessment, one could not determine whether there are differences in *reactivity* between conditions (i.e., differences in the magnitude of increases in cortisol, which can be examined if a response is elicited) or whether there are differences in cortisol *levels* (i.e., differences in naturally-declining circadian values). Therefore, regardless of the experimental question, a baseline pre-stressor cortisol assessment should be obtained.

Given the importance of this pre-stressor sample, efforts should be made to ensure the quality and integrity of this assessment. There are some restrictions (e.g., caffeine intake, exercise, meals) which can be implemented so that these external inputs do not influence participants' baseline assessments (for details, see "Health Behaviors" section below). Additionally, most studies employ a rest period in the laboratory prior to obtaining a baseline sample. This is important because there is a time lag between external events and cortisol elevations of approximately 20–40 minutes. Therefore, cortisol levels upon entering the lab really best reflect what happened to the individual about 30 minutes beforehand. For example, if a participant experienced a stressor on the way to their scheduled session, this could manifest as a cortisol elevation approximately 30 minutes later. A rest period allows the HPA system to return to resting baseline levels, irrespective of external events. Additionally, a rest period provides a consistent, standardized period for all participants prior to the stressor, which could reduce variability in baseline assessments that increase statistical power and lead to cleaner, more interpretable data.

The length of the baseline rest period significantly predicted effect sizes in a meta-analysis of cortisol reactivity (Dickerson & Kemeny, unpublished observations), where longer rest periods were associated with larger cortisol responses. This suggests that an adequate rest period is important for the elicitation and/or capture of a strong cortisol response. Many studies employ at least a 10–40 minute rest period prior to the onset of a stressor when assessing salivary cortisol (e.g., Kirschbaum et al., 1993; Dickerson et al., 2008) that has proven adequate for observing reliable cortisol responses. Longer rest periods may be required when plasma cortisol is assessed in order to allow the participant to fully recover from venipuncture (e.g., 45–60 minutes; Lovallo & Thomas, 2000).

Reactivity Timing of assessment is also critical for capturing peak cortisol responses to a psychosocial stressor in the laboratory. In a meta-analytic review

of 208 acute psychological stressor studies (Dickerson & Kemeny, 2004), cortisol reached peak levels on average 20–40 minutes after the onset of a psychosocial stressor. Effect sizes for increases in cortisol obtained 20–40 minutes from stressor onset ranged from d = 0.38–0.41; those from samples obtained less than 20 minutes or more than 40 minutes from stressor onset were considerably smaller (d's = 0.13–0.29). It should be noted, however, that effect sizes for the samples obtained 1–10 minutes from stressor onset were also significantly greater than zero, indicating that stress-induced changes in this system could be observed relatively quickly. However, peak increases typically fell during this 20–40 minute period from stressor onset. Therefore, assessing cortisol during the 20–40 minute window would maximize the chances of capturing peak cortisol changes.

Recovery Timing is also important when assessing cortisol recovery, or how long it takes for cortisol values to return to resting baseline or pre-stressor levels. The meta-analysis (Dickerson & Kemeny, 2004) also examined cortisol recovery processes. Effect sizes were calculated for cortisol samples obtained 0–20 minutes, 21–40 minutes, and 41–60 minutes from stressor termination. The largest effect sizes were seen in samples obtained 0–20 minutes from the end of a stressor (d = 0.38), followed by 21–40 minutes (d = 0.26). These effect sizes were significantly different from zero, indicating that cortisol levels were, on average, still elevated up to 40 minutes post-stressor. However, the effect size for samples obtained 41–60 minutes from stressor onset was not significant (d = -0.05), demonstrating that cortisol levels had typically returned to baseline levels by 40–60 minutes post-task.

However, recovery was significantly predicted by the type of task utilized. Cortisol elevations were still observed during the 41–60 minute period for social-evaluative, uncontrollable stressors; cortisol levels had returned to baseline levels for studies utilizing stressors without both characteristics (for details, see "Uncontrollable, Social-Evaluative Threat" section below). This indicates that task selection could also be an important factor to determine how long to follow participants after a psychosocial stressor challenge to adequately assess recovery processes.

Based on these findings, investigators interested in charting patterns of cortisol recovery should obtain samples for at least 60 minutes after stressor termination (to allow the majority of participants to return to baseline). However, this window should be extended past the 1 hour post-stressor mark for tasks that strongly stimulate the HPA system (e.g., TSST or other social-evaluative, uncontrollable tasks).

Number The optimal number of assessments and timing of samples is dependent on the specific research question. At minimum, studies examining reactivity should assess cortisol at two time points: baseline and post-stressor (ideally, 20–40 minutes from stressor onset to capture maximum increases). However,

assessing cortisol repeatedly could lead to more fine-grained analyses and subtle distinctions between individuals and/or conditions. This could be particularly important if employing a subtle manipulation with the expectation of small differences between conditions. Additionally, if one is interested in examining recovery, periodic assessment at least through the 40–60 minute period post-stressor or beyond is necessary to allow the majority of participants to return to baseline.

ACTH responses In addition to examining cortisol, some laboratory studies also assess ACTH responses. Such an assessment helps determine how different components of the HPA axis may function may converge or diverge as in the context of acute stressors. Compared to cortisol, ACTH is released earlier in the HPA cascade, and therefore has a parallel – but distinct – trajectory. This has important implications for the timing of assessments of this hormone in HPA research.

The Dickerson & Kemeny (2004) meta-analysis examined a sub-set of studies (k = 39) that assessed both ACTH and cortisol responses to psychological stressors. Overall, ACTH responses predicted cortisol responses; nearly equivalent effect sizes were observed for overall increases in ACTH and cortisol in response to psychological stressors, indicating that the magnitude of these stress-induced changes were similar. Although individual differences can sometimes predict dissociations at different levels of the HPA axis (e.g., depression; Gold, Licinio, Wong & Chrousos, 1995), these findings suggest that in general, ACTH and cortisol are elicited in parallel among healthy individuals.

However, the timing of the increases in ACTH and cortisol showed different patterns. Peak increases in ACTH were observed 11–20 minutes from stressor onset, with levels showing a linear decline throughout the subsequent 21–60 minutes. Therefore, peak ACTH responses preceded peak cortisol responses by approximately 10–20 minutes. This divergence between peak levels of ACTH and cortisol suggest that multiple assessments should be made if multiple indicators of HPA activity are assessed (e.g., one assessment 15 minutes from stressor onset would capture maximal ACTH changes, but miss maximal cortisol changes, which would occur approximately 10–20 minutes later). Therefore, repeated sampling is important to capture peak changes in both hormones.

Sampling

Cortisol levels can be assessed in plasma, saliva, or urine. However, urinary cortisol is not optimal for laboratory reactivity studies because it is not as sensitive to short-term changes in response to acute stressors; urinary samples may be most appropriate when one wants an integrated picture of HPA activity over an extended sampling time (e.g., 24 hours; Lovallo & Thomas, 2000). Therefore, only plasma and saliva sampling will be discussed further in the context of laboratory reactivity protocols.

Cortisol can be either bound to the proteins albumin or transcortin (also known as corticosteroid-binding globulin, or CBG), or unbound in a biologically active form. In blood, approximately 3–5% of cortisol is free, while the remainder is bound to CBG. Thus, cortisol assessed through blood samples represents total cortisol – both the free and bound fractions. In contrast, salivary cortisol represents only the unbound or biologically active fraction. Thus, selection of the optimal method of assessment (plasma versus saliva) should be driven, in part, by the research question, and whether total or unbound cortisol assessment is most relevant for addressing study goals.

Either salivary or plasma samples can be used to reliably assess cortisol reactivity. Obtaining plasma samples requires a trained phlebotomist, nurse, or physician to implement the blood draws (either by venipuncture or placement of an indwelling catheter) as well as careful compliance with special safety precautions for the handling and storage of blood. Plasma samples are necessary if one wants to assess total cortisol (bound and unbound); and, ACTH cannot currently be assessed through saliva, and so incorporating this hormone within a study protocol would necessitate plasma samples.

There has been a surge in studies that have utilized salivary assessment, due to its ease and convenience. Salivary cortisol is not affected by saliva flow rate (Kirschbaum & Hellhammer, 1989, 1994), making it a reliable assessment method in the context of laboratory stressor studies. Saliva samples are most commonly obtained with the use of a Salivette (Sarstedt, Newton, NC; www.sarstedt.com), a small plastic tube that contains a cotton roll.[1] Participants place the cotton in their mouths for 2–3 minutes, then place it back into the plastic tube. Thus, saliva sampling can be self-administered and is appropriate for use in both laboratory and home settings. Although salivary cortisol is stable at room temperature for up to two weeks, salivettes should be placed in a freezer (ideally at temperatures of −20° C or colder) as soon as possible after collection.[2]

Typically, plasma and salivary cortisol levels are highly correlated (*r*'s > .90; Kirschbaum & Hellhammer, 1989, 1994). In a meta-analytic review of acute psychological stressor studies, method of cortisol assessment (plasma versus saliva) was not a significant predictor of effect sizes; in other words, method of assessment was unrelated to the magnitude of the cortisol changes. This suggests that, on average, salivary and plasma assessment result in similar patterns of cortisol reactivity. However, there are conditions under which the patterns may diverge. Specifically, there are factors that can influence CBG (e.g., fluctuations in estrodial; see "Gender" and "Hormonal Status in Women" sections below), which in turn can alter the bound fraction of cortisol represented in plasma samples. Therefore, in some cases saliva and plasma samples can result in different patterns of cortisol reactivity, since these methods assess different cortisol fractions (i.e., bound versus total cortisol).

Task Characteristics

Selection and implementation of the appropriate stressor in cortisol research can be critical for effectively eliciting this parameter. There is a great deal of variability in the types of tasks that have been used in the literature, as well as a great deal of variability in their effectiveness in eliciting HPA activity. Careful attention to the type of task selected, as well as the execution of specific contextual details, can be important determinants of whether cortisol increases in the context of laboratory reactivity studies.

Relative effectiveness of different types of tasks

A number of different types of stressors have been utilized in cortisol reactivity research (e.g., performing difficult cognitive tasks, delivering speeches, discussing marital problems, and watching distressing films). The Dickerson & Kemeny (2004) meta-analysis demonstrated that a great deal of the variability in cortisol responses to psychological stressors can be explained by the type of task used; certain categories of stressor tasks consistently and strongly elicit cortisol responses, whereas others fail, on average, to activate this system.

The meta-analysis grouped the stressors used in this literature into five different categories in order to judge their relative effectiveness for activating the HPA system. *Cognitive tasks* included stressors that require participants to perform arithmetic calculations, complete a stroop color-conflict task or test of perceptual skill (i.e., mirror tracing), or answer questions that test verbal, analytical, or quantitative abilities. *Verbal interaction tasks* included public speaking tasks, interviews, or discussion of problems within a marital relationship. *Public speaking/cognitive task combination tasks* required participants to complete both a public speaking and cognitive task in sequence; a commonly-used example of in this stressor category is the Trier Social Stress Test (TSST: Kirschbaum et al., 1993). *Emotion induction tasks* included having participants watch a distressing film, write about negative experiences, or be exposed to other emotion-eliciting material. With *noise exposure tasks*, participants experienced intermittent or continuous loud noise (without an accompanying cognitive task). Analyses controlled for time of day and timing of cortisol assessment, and so the findings were independent of these methodological considerations.

Only certain stressor categories were capable of elevating cortisol levels. Overall, the emotion induction tasks and noise exposure tasks failed to increase cortisol levels (effect size d = -0.13, d = -0.06, respectively). Cognitive tasks and verbal interaction tasks did elicit a significant cortisol responses; however, the effect sizes for these stressor categories were relatively small (d = 0.20, d = 0.39, respectively). The most potent task in terms of triggering strong increases in cortisol was the public speaking/cognitive task combination (e.g., TSST), with an effect size of d = 0.89

(considered a large effect). Overall, this demonstrates that not all stressors elicit cortisol responses, and certain tasks are much more effective than others in activating this system, particularly those categorized by Obrist (1981) as active (i.e., cognitive, verbal interaction, and cognitive/speaking) rather than passive coping tasks (i.e., watching a scary movie). Further, it highlights that stressor category is important to consider when designing studies to elicit cortisol reactivity.

Trier social stress test

The Trier Social Stress Test (TSST) is an example of a public speaking/cognitive combination task for adults. Its effectiveness in eliciting strong and meaningful increases in cortisol (e.g., Dickerson & Kemeny, 2004; Kirschbaum et al., 1993) as well as its prevalence in the literature warrants a more in-depth description. Although several researchers had already used public speaking and serial subtraction in their research studies, the TSST was developed by Kirschbaum, Pirke, and Hellhammer to standardize and strengthen the protocol. It is described in their publication (1993) that outlines the protocol in detail.

First, participants arrive in the laboratory for individual appointments, and complete a rest period (10–40 minutes). Then, they are escorted to another room in which they are given the task instructions. Three individuals are seated behind a table, with a video-camera and tape recorder already set up. Participants are instructed to pretend that they are a job applicant interviewing for a position in a company. They are told that they should convince the panelists that they would be the perfect person for the position in a 5-minute free speech; voice frequency and non-verbal behavior analyses would also be performed with the video- and audio-tapes. After hearing the instructions, participants return to the original room and are given 10 minutes to prepare their speeches.

After the 10-minute preparation period, participants are led back into the room and instructed to stand in front of a microphone. They then begin their 5-minute speech in front of the panelists. The panelists provide standardized verbal prompts if the participant stops speaking, and maintain stoic, serious expressions throughout the task. Following the speech, the participants are asked to subtract the number 13 from 1022 as quickly and accurately as possible. If the participants answer incorrectly, they are stopped and asked to begin again. After 5 minutes, the participants are asked to stop and are escorted back to the original room, where they provide additional saliva/plasma samples throughout a recovery period (typically 45–70 minutes from the end of the task) and are debriefed regarding the study aims. This protocol has been shown to elicit two- to four-fold increases in cortisol from baseline values in the majority of participants (Kirschbaum et al., 1993).

TSST modifications Since their 1993 publication, this research group has made several modifications to the TSST protocol. First, the stressor has been shortened

to 15 minutes (rather than 20 minutes); 5 minutes are allocated for task instructions/speech preparation (changed from the original 10 minutes), 5 minutes for the speech delivery, and 5 minutes for the math task. Second, the number of panelists present during the speech and math components has been reduced from three to two. Finally, the participants now prepare their speech in the room in which it is delivered (rather than going back to the original room during this time). These changes do not appear to be associated with the tasks' ability to elicit strong cortisol responses; this modified version is capable of triggering robust cortisol increases.

Other research groups have also modified certain details of the TSST, and demonstrated that slightly modified forms of this task can still elicit a strong cortisol response. For example, some studies that have had participants remain in the same room and/or remain seated throughout the laboratory session (and the panelists enter and exit when needed) have shown significant increases in cortisol; this modification of the original TSST may be particularly important for studies that are also assessing cardiovascular reactivity (see Chapter 2). There has also been some variability in speech topics; while the original form of the TSST has participants talk about why they would be a good job candidate, others have instructed participants to discuss a controversial topic or defend oneself against a false accusation of shoplifting (e.g., al'Absi, Bongard, Buchanan, Pincomb, Licinio, & Lovallo, 1997; Cohen, Hamrick, Rodriguez, Feldman, Rabin, & Manuck, 2000), talk about their strengths and weaknesses (e.g., Van Eck, Nicolson, Berkhof, & Sulon, 1996), or a negative life experience (e.g., Abplanalp, Livingston, Rose, & Sandwisch, 1977). Overall, it appears the speech topic is less important than giving the speech itself; the social-evaluative nature of the speech performance seems to be the critical factor that triggers cortisol.

However, it should be noted that few studies have experimentally manipulated these components (e.g., whether participants are seated versus standing, topic of the speech) and so it is not clear whether these factors would moderate cortisol reactivity or lead to small differences in magnitude of the response. However, varying these procedural details does not seem to have a dramatic impact on whether cortisol response is capable of being elicited.

A modified version of the TSST has been shown to effectively elicit cortisol responses in children (TSST-C; Buske-Kirschbaum, Jobst, Wustmans, Kirschbaum, Rauh, & Hellhammer, 1997). The protocol is quite similar to the adult version described above; however, rather than delivering a speech on why they would be a good job applicant, the children receive the beginning of a story as a prompt. They are then asked to complete it in as exciting and interesting way as possible, and to try to perform better than other kids on the task (see Buske-Kirschbaum et al., 1997, for details of this procedure). Additionally, children also solve easier mental arithmetic problems than with the adult version, depending on their age (e.g., counting backwards by 7s rather than 13s). The most important distinction

between the two versions, however, is that with the TSST-C, the panelists are instructed to provide positive verbal and facial feedback (in contrast to the stoic or neutral feedback that is delivered in the adult version). The TSST-C has been shown to elicit strong, significant cortisol increases in the majority of children (Buske-Kirschbaum et al., 1997), albeit at a slightly reduced magnitude (approximately 30%-50% lower) compared to the adult version of the TSST. However, other studies have modified the TSST-C and shown similar responses in children and adults (Yim, Quas, Cahill, & Hayakawa, 2010). TSST-C has now been utilized in many studies, and nearly all have found that it increases cortisol levels (for extensive review, see Gunnar, Talge, & Herrera, 2009).

Task characteristics that predict cortisol responses

It is clear that the TSST – in its original and modified forms – can elicit strong cortisol responses. What is it about the task that leads to these changes? Findings from the Dickerson & Kemeny (2004) meta-analysis demonstrate that the TSST is effective because it has elements of uncontrollability and social-evaluative threat – key factors that predict cortisol reactivity in the laboratory.

Uncontrollability Uncontrollable contexts have long been posited to lead to elevations in cortisol. There is clear support from research in non-human animals that uncontrollable stressors lead to greater increases in cortisol, compared to relatively controllable stressor conditions. For example, animals that lack control over blasts of noise or electric shocks typically show elevated cortisol levels compared to yoked animals who receive the same stimuli but have control (e.g., can push a lever to stop the noise/shock; Dess, Lindwick, Patterson, Overmier, & Levine, 1983; Swenson & Vogel, 1983; Weiss, 1971). However, findings from studies in humans which have manipulated uncontrollability have been mixed; some have found that uncontrollability leads to greater cortisol responses (Breier, 1989; Peters et al., 1998) whereas others have not (Bohlin, Eliasson, Hjemdahl, Klein, & Frankenhauser, 1986; Steptoe, Fieldman, Evans, & Perry, 1993).

The Dickerson and Kemeny (2004) meta-analysis tested whether, across the 208 acute laboratory stressor studies, uncontrollability was a significant predictor of cortisol reactivity. Stressors were coded as "uncontrollable" if behavioral responses could not affect outcomes, or nothing could be done to change the situation (e.g., Levine & Ursin, 1991; Weiner, 1992). This included if: a) task difficulty was manipulated (e.g., participants performed under time constraints or attempted impossible tasks); b) false feedback regarding performance was given; c) participants received criticism or harassment from an experimenter; or d) there was presence of continuous noise, distraction, or emotional stimuli. These elements were considered to induce relatively uncontrollable conditions, as they create contexts of forced failure where there is no behavioral response possible.

The meta-analytic results demonstrated that uncontrollable stressors were associated with larger cortisol responses ($d = 0.52$) compared to relatively controllable ones ($d = 0.16$). Thus, these findings are aligned with the research in non-human animals, which has found that uncontrollability is an important stressor dimension associated with cortisol reactivity. Therefore, employing tasks with uncontrollable elements in cortisol reactivity studies may maximize the chance of eliciting strong cortisol responses.

Social-Evaluative Threat (SET) Another element that has been proposed to elicit cortisol responses is *social-evaluative threat* (SET), which occurs when an important aspect of the self could be negatively judged by others (Dickerson & Kemeny, 2004). This is grounded in the premise that humans have a fundamental need to belong and be socially accepted by others (e.g., Baumeister & Leary, 1995); therefore, situations that jeopardize this goal could be perceived as a profound threat, capable of eliciting psychobiological responses. Indeed, research in non-human primates and other animals has demonstrated that threats to social status are associated with elevated HPA activation (e.g., Sapolsky, 2005; Shively, Laber-Laird, & Anton, 1997). In humans, social-evaluative threat within a laboratory context includes experiences when poor performance could reveal a lack of important characteristics or abilities (e.g., intelligence, competence), resulting in the potential for a loss of social esteem, status, or acceptance among others. Elements of the stressor that could induce social-evaluative threat include: a) the presence of an evaluative audience; b) the presence of a negative social comparison; or c) video- or audio-recording of the performance (that creates the potential for subsequent evaluation).

The Dickerson and Kemeny (2004) meta-analysis demonstrated that stressors with social-evaluative elements triggered larger increases in cortisol ($d = 0.67$) compared to tasks without these components ($d = 0.15$). Additional analyses demonstrated that real-time social evaluation may be more effective than the potential for subsequent evaluation; effect sizes for tasks with an audience or negative social-comparison were larger than the tasks that were video- or audio-taped. This suggests that including the physical presence of evaluative others or real-time social evaluation into experimental protocols may be a very effective method of triggering strong cortisol responses in the laboratory.

Experimental studies have further underscored social-evaluative threat as a potent elicitor of strong cortisol responses. Gruenewald and colleagues (Gruenewald, Kemeny, Aziz, & Fahey, 2004) randomly assigned participants to undergo a modified version of the TSST in one of two conditions: alone in a room (non-SET) or in front of a 2-member audience panel and video camera (SET). Results demonstrated that only the SET condition elicited cortisol reactivity; there were no increases in cortisol when the identical task was performed in the absence of social evaluation. Several recent studies have replicated this finding (Dickerson et al.,

2008; Het, Rohleder, Schoofs, Kirschbaum, & Wolf, 2009); both found no cortisol reactivity following a speech and/or math task performed alone, and robust increases when performed in front of an evaluative audience. Similar findings have also been obtained using physical stressor (e.g., cold pressor task; Schwabe, Haddad, & Schachinger, 2008) and naturally occurring performance stressors (e.g., ballroom dancing competition; Rohleder et al., 2007). Taken together, these studies provide strong evidence that social-evaluative threat provides one condition capable of eliciting cortisol responses, and is a vital element to include when designing protocols to activate this system.

Uncontrollable, social-evaluative threat Given that both social-evaluative threat and uncontrollability predicted cortisol reactivity in the Dickerson and Kemeny (2004) meta-analysis, additional analyses explored the relationship between these constructs, and whether uncontrollable, social-evaluative threat would have an additive effect on the HPA axis. The stressor tasks were grouped into those that had both elements, those that had one element, and those that did not have either element. Findings demonstrated that tasks without either uncontrollability *or* social-evaluative threat did not activate this system; the effect sizes were not significantly different from zero among this stressor category (*d*'s ranged from –0.08 to –0.07). Tasks with one element did elicit a significant cortisol response; however, tasks with both elements were associated with more than two times the effect size of either component alone ($d = 0.92$, for uncontrollable, social-evaluative tasks versus $d < 0.40$ with one component). Additionally, uncontrollable, social-evaluative threat triggered larger increases in ACTH compared to tasks without both elements. Uncontrollable, social-evaluative threat creates conditions of exposed failure, in which others can observe poor performance. Uncontrollability can therefore heighten the effect of social-evaluative threat on HPA reactivity. Incorporating both elements into experimental protocols is likely to maximize cortisol elevations.

Furthermore, the meta-analysis demonstrated that the public speaking/cognitive combination task (e.g., TSST) is effective at eliciting cortisol levels, because it includes both social-evaluative threat and uncontrollability. When type of task, social-evaluative threat, and uncontrollability were simultaneously entered into a regression equation, social-evaluative threat and uncontrollability remained significant predictors of effect sizes, whereas type of task was no longer significant. Analyses showed that social-evaluative threat and uncontrollability fully mediated the effect of type of task on cortisol reactivity; in other words, the reason that the TSST and related tasks are capable of eliciting strong, significant cortisol responses is because of the social-evaluative and uncontrollable elements. This highlights the importance of not only what category of task is selected for experimental protocols, but that including the proper social-evaluative and/or uncontrollable elements in these tasks is crucial for eliciting cortisol responses.

Specific social-evaluative elements associated with cortisol reactivity There is a lot in a SET context that makes it different from a non-SET context. For example, when people are present, they often negatively evaluate the participant and there is often the potential for future evaluation (via video- or audio-tape). A series of studies have tried to "unpack" the social-evaluative threat effect, in order to identify the active ingredient in this context that is capable of eliciting cortisol responses. These studies have methodological implications for employing the specific components of the SET context that are necessary to trigger cortisol reactivity.

Dickerson and colleagues (2008) tested whether social presence is a sufficient condition to elevate cortisol levels, or whether observers must be in an evaluative mode to trigger changes in this system. Participants were randomly assigned to deliver a speech in one of three conditions: 1) alone in a room (non-SET), 2) in front of a 2-member evaluative audience panel, or 3) an inattentive presence condition, where someone was present in the room during the speech, but was working on a computer and not paying attention to the participant during the task. Results demonstrated that the non-SET and inattentive presence condition did not elicit a cortisol response; increases in this hormone were only observed for those delivering a speech in front of an evaluative audience. These findings suggest that it is not the mere social presence of others that elicits cortisol responses. Further, it indicates that protocols designed to elicit cortisol reactivity should not only have others present, but be present in an evaluative mode.

Other studies have examined whether the visible physical presence of others is necessary to elicit cortisol reactivity, or whether panelists could ostensibly be evaluating the participant from another location (i.e., through a one-way mirror). Elevations in cortisol levels have been found in studies in which remote evaluation occurred via a one-way mirror or television screen (Andrews, Wadiwalla, Juster, Lord, Lupien, & Pruessner, 2007; Jansen, Wied, & Kahn, 2000; Kelly, Matheson, Martinez, Merali, & Anisman, 2007; Kemmer et al., 1986; but see Lupien et al., 1997), intercom (Het et al., 2009), or virtual audience (Kelly et al., 2007). However, the increases observed for these remote evaluative conditions have generally been smaller than those found for a "live" audience (Het et al., 2009; Kelly et al., 2007; but see Andrews et al., 2007). For example, Kelly and colleagues (2007) found that remote evaluation (experienced virtually or via a one-way mirror) resulted in increases of approximately 25% over baseline levels, whereas performing in the presence of an evaluative audience resulted in increases of approximately 90%. Together, this suggests that a "live" audience may augment and heighten cortisol reactivity, but creating remote social-evaluative contexts can also trigger this system (albeit at potentially lower levels).

Research has also examined whether the number of evaluators matters in terms of eliciting cortisol reactivity. The presence of one panelist appears to be enough to trigger cortisol responses (e.g., Andrews et al., 2007; Bosch et al., 2009); studies that have utilized one evaluative audience member during performance tasks

have demonstrated increases in cortisol. One study found no differences in cortisol responses when participants were randomly assigned to perform in front of one audience member versus two (Andrews et al., 2007). However, this study only examined men, and so it is unclear whether this same effect would be observed among women. Another study found that an audience composed of four members elicit significantly greater cortisol responses than an audience of one (Bosch et al., 2009), suggesting that multiple panelists may lead to larger increases in cortisol. Future research should continue to test the effects of the number of panelists on cortisol reactivity.

Other studies have examined whether the potential for future evaluation provides a sufficient condition to elicit cortisol reactivity, or whether others must be present to trigger these changes. Robbins and colleagues (Robbins, Dickerson, Epstein, & Zaldivar, under review) found that having participants deliver a speech alone in a room – but with a video camera recording the performance – did not increase cortisol responses. In fact, there were no significant differences between the videotape context and a non-SET context (without a video camera) in terms of cortisol trajectories; neither elicited cortisol increases. This demonstrates that the potential for future evaluation – in the absence of other social-evaluative cues – does not activate this system. Further, it suggests that conditions that only videotape stressor performances may not adequately elicit changes in cortisol; others may need to be present in an evaluative mode to activate this system.

Some studies have had given participants instructions for a speech, allowed them time to prepare, and then told them that they would not have to deliver the speech after all – thus, there is anticipatory social evaluation, but the participants do not ever go through the task. The results of these studies have been mixed; some have shown that anticipation of a stressor can increase cortisol levels (e.g., Starcke, Wolf, Markowitsch, & Brand, 2008), while others have shown no changes (e.g., Kelly et al., 2007; Rohrmann, Hennig, & Netter, 1999). When increases have been observed, they have tended to emerge for only a subset of individuals (e.g., men but not women; Kirschbaum, Wust & Hellhammer, 1992); and the increases in cortisol have generally been much smaller in magnitude compared to protocols in which the speech is actually delivered. These findings suggest that anticipation of social evaluation may elicit small increases in cortisol among some individuals, but may be a less reliable way of activating the HPA axis compared to actually delivering a speech in front of an evaluative audience.

Social-evaluative threat: Conclusions and methodological implications The results from a growing number of studies that have investigated social contexts and cortisol reactivity have identified several characteristics of stressors that should be implemented to elicit maximum cortisol responses. To achieve the largest cortisol responses, it appears one would want to select a task with uncontrollable and social-evaluative elements, and specifically, have the panelists physically present

and in an explicitly evaluative mode. However, one might not always want to achieve this maximum increase in cortisol reactivity; tasks that more moderately activate the HPA axis may be sufficient in the context of many studies. Those with either uncontrollability or social evaluative threat, or those with a remote audience, could serve the purpose of eliciting moderate cortisol elevations. However, tasks that lack social-evaluation and uncontrollability or are only videotaped, or where others are not in an evaluative mode do not appear capable of eliciting a cortisol response.

Novelty Novelty has been long-proposed as a dimension linked with cortisol reactivity (e.g., Mason, 1968). Most of the studies examining cortisol reactivity in the laboratory have participants complete the task during one experimental session; thus, the stressor is relatively novel for many of the participants, and could be an element linked with cortisol elevations. Indeed, studies that have brought participants back into the lab for multiple sessions and repeated the stressor (e.g., performed the TSST three times in one week) typically have found reductions in cortisol responses on subsequent sessions (e.g., Cohen et al., 2000; Kirschbaum et al., 1995a; Schommer, Hellhammer, & Kirschbaum, 2003); this suggests that novelty can impact cortisol responses. However, most studies have found significant (albeit reduced) increases in cortisol on subsequent exposures, indicating that novelty is not the only factor driving cortisol responses. Additionally, it could be that uncontrollability and novelty are linked; for example, novel stressors may be perceived as less controllable, or experience with a task may lead to reductions in appraisals of uncontrollability.

Individual differences in novelty – or experience with a given stressor task – could also potentially influence cortisol responses. For example, one's experience and/or comfort with public speaking could be associated with the elicitation and/or magnitude of the cortisol response. Individual differences in task experience would be worth assessing via questionnaire prior to task initiation in the context of some studies.

Stressor length Laboratory stressors can widely vary in terms of length; some can last just a few minutes, whereas others can go on for several hours. The Dickerson and Kemeny (2004) meta-analysis examined whether the length of a stressor predicted cortisol reactivity. Overall, stressor length was not associated with effect sizes; in other words, the duration of the stressor was not associated with the magnitude of the stressor response. Relatively short stressors seem to be quite effective in eliciting cortisol responses, provided that they have elements of uncontrollability and/or social-evaluative threat. However, few experimental studies have manipulated task length to examine its effects on cortisol reactivity, and it is possible that this could moderate the responses. Taken together, though, it appears that stressor length is not a primary dimension related to cortisol reactivity in the laboratory.

Tasks to assess individual differences in cortisol reactivity The previous discussion focused on identifying tasks that elicit strong, significant cortisol responses in the majority of individuals; although tasks like the TSST have been shown to effectively induce large cortisol changes, this does not mean that this is the *only* type of task that should be utilized in cortisol research. Achieving maximal cortisol responses might not be the goal of a given study or program of research. Some research questions may best be pursued with tasks that only a portion of participants respond to, but allow the examination of individual differences that predict reactivity in a specific context. For example, marital conflict studies have typically shown that overall, discussing a problem in one's marriage does not lead to elevations in HPA parameters (e.g., Kiecolt-Glaser et al., 1997; Malarkey, Kiecolt-Glaser, Pearl, & Glaser, 1994; Miller, Dopp, Myers, Stevens, & Fahey, 1999). However, those high on cynical hostility (Miller et al., 1999), those who receive hostile behaviors (Kiecolt-Glaser et al., 1997; Malarkey et al., 1994) or are insecurely attached (Powers, Pietromonaco, Gunlicks, Sayer, 2006) have shown elevations in HPA activity. This task has been effective for identifying theoretically-derived subsets of individuals who may be particularly vulnerable in this stressor context. Therefore, it is important to note that just because a task, on average, does not activate the HPA system, it is not the case that it should never be used in this area of research. On the contrary, there can be certain situations in which these tasks can illuminate individual differences that might not emerge in other contexts. For example, relationship-oriented variables (e.g., attachment) may be particularly relevant when examining relationship conflict as a stressor, and might not significantly predict reactivity in other stressor contexts (e.g., mental arithmetic). Thus, matching the appropriate stressor task with the appropriate research question is of paramount importance.

Individual Differences and Cortisol Research

There are a number of individual differences that can influence the elicitation and magnitude of cortisol responses to acute laboratory stressors. Researchers should be aware of associations between cortisol responses and demographic factors, hormonal states, and health behaviors when deciding on exclusion criteria, behavioral restrictions, and assessments in laboratory investigations.

Gender

There have been a number of studies that have examined gender differences in cortisol reactivity to acute laboratory stressors, and several comprehensive review papers have been published on this topic (e.g., Kudielka & Kirschbaum, 2005; Kajantie & Phillilps, 2006). Studies have explored biological and psychological explanations for potential gender differences in physiological reactivity.

In the Dickerson & Kemeny (2004) meta-analysis, gender was examined as a predictor of cortisol responses. For each study, the gender composition of the sample was coded as "percent male" to assess the ratio of men to women in the study. This ratio was not a significant predictor of effect sizes; in other words, the gender composition of the sample did not explain a significant amount of variability in cortisol responses across the studies. This indicates that men and women may generally show similar cortisol responses to acute stressors; however, this gender ratio measure was not very sensitive and did not provide a sophisticated test of this hypothesis.

A number of studies have directly compared men's and women's cortisol responses to standardized acute stressors, such as the TSST. Across a number of studies, men have shown greater cortisol reactivity to psychosocial laboratory stressors compared to women (although there has been some variability in these effects; see Kudielka & Kirschbaum, 2005; Kajantie & Phillips, 2006; Otte, Hart, Neylan, Marmar, Yaffe, & Mohr, 2005, for review). Typically, no differences in resting or baseline cortisol levels have been observed; the divergence in cortisol seems to appear primarily after psychosocial challenge. Additionally, it should be noted that in the majority of these studies, increases in HPA activity (ACTH, salivary cortisol, or plasma cortisol) were seen in response to stressors in both genders. In other words, these parameters were elevated following a psychosocial stressor among both men and women, although there were often differences in the magnitude of these increases.

There is some evidence that men and women may respond to different types of stressors. For example, Stroud and colleagues (Stroud, Salovey, & Epel, 2002) found that men show greater cortisol reactivity to achievement-based tasks (i.e., mental arithmetic), whereas women show greater reactivity to interpersonal tasks (i.e., social rejection). Consistent with the premise that women may be more sensitive to interpersonal stressors than men, Kiecolt-Glaser and colleagues (Kiecolt-Glaser et al., 1997; Malarkey et al., 1994) have shown that women are more responsive to hostile interpersonal behaviors in marital relationships. Taken together, these findings suggest that interpersonal stressors may be more likely to elicit cortisol reactivity among women. However, this question requires additional investigation.

Whether gender differences in cortisol reactivity are present appears to depend, in part, on the method of cortisol assessment and the hormonal milieu of the female participants. For example, in an elegant study, Kirschbaum and colleagues (Kirschbaum, Kudielka, Gaab, Schommer, & Hellhammer, 1999) found that men showed larger increases in ACTH and salivary cortisol compared to women; however, there were no differences between genders for plasma cortisol. Furthermore, the salivary cortisol response among females depended on oral contraceptive use and menstrual cycle phase, suggesting that hormonal factors may account for a portion of the variability in cortisol responses between genders (as addressed below).

Methodological implications Given that gender has been consistently shown to moderate cortisol reactivity, it is important to balance the gender composition of the sample as much as possible, and have a sufficient sample size to either control for gender or examine it as a potential moderator in analyses. In experimental studies in which participants are randomly assigned to conditions, it could be beneficial to block by gender to ensure equal representation of men and women across conditions. Additionally, the selection of the task could be particularly important when designing cortisol reactivity studies when examination of gender differences is a primary research question.

Hormonal status in women

A number of studies have demonstrated that menstrual cycle phase or the use of oral contraceptives can have a significant impact on cortisol reactivity; as both are associated with pronounced changes and/or alterations in reproductive hormones (e.g., estradial) that can influence HPA activity. The menstrual cycle lasts, on average, 28 days, and can be divided into several phases. Menstrual bleeding typically occurs on days 1 through 4 of the cycle. This is followed by the follicular phase (days 5–13) that is associated with increasing levels of estradial. Ovulation (when the egg is released) typically occurs on day 14, and is followed by the luteal phase (days 15–28) that is characterized by relatively low levels of estradial.

Several studies have demonstrated that *salivary* cortisol responses are sensitive to menstrual cycle phase. Specifically, women in the luteal phase of the menstrual cycle have shown significantly greater cortisol responses compared to women in the follicular phase (e.g., Kirschbaum et al., 1999; Rohleder, Schommer, Hellhammer, Engel, & Kirschbaum, 2001; Wolf, Schommer, Hellhammer, McEwen, & Kirschbaum, 2001). In fact, women in the luteal phase have shown comparable cortisol responses to men, suggesting that menstrual cycle phase may explain some of the observed differences in cortisol reactivity between genders. It is important to note that typically resting or basal cortisol values have not varied according to menstrual cycle phase; only when undergoing a psychosocial challenge have differences emerged.

Studies have also shown that oral contraceptive use is associated with salivary cortisol reactivity. Specifically, women on oral contraceptives (OC) have shown blunted salivary cortisol responses compared to men (e.g., Kirschbaum, Pirke, & Hellhammer, 1995b; Kirschbaum et al., 1999) and women not on OC medication (e.g., Kirschbaum et al., 1995b). Subsequent studies compared women on OC medication and control women during different phases of the menstrual cycle. Women on oral contraceptives have shown blunted salivary cortisol reactivity relative to women in the luteal phase (Kirschbaum et al., 1999; Rohleder, Wolf, Piel, & Kirschbaum, 2003); however, no differences were found between women

on OCs and those in the follicular phase of the menstrual cycle (Kirschbaum et al., 1999).

Differences between women on oral contraceptives and in different phases of the menstrual cycle have emerged when examining *salivary* cortisol. In contrast, there generally are no differences in terms of *plasma* cortisol reactivity (e.g., Abplanalp et al., 1977; Kirschbaum et al., 1999). Plasma cortisol represents cortisol bound to the corticosteroid-binding globulin (CBG) protein as well as free cortisol (unbound cortisol); thus, it is a measure of total cortisol. However, salivary cortisol represents only levels of free cortisol, and not the bound fraction. Given that estrogen can increase CBG (Moore, Kawagoe, Davajan, Nakamura, Mischell, 1978), this could affect the availability of biologically-active or free cortisol, leading to blunted salivary responses; however, total cortisol (represented by plasma cortisol) appears to be less affected by hormonal status (i.e., menstrual cycle phase and/or hormonal contraceptive use.

Methodological implications Given reliable differences in salivary cortisol reactivity associated with different phases of the menstrual cycle, it may be optimal to schedule laboratory sessions so that all women are in a single phase (e.g., all in the luteal phase). However, this may be time- and cost-prohibitive in the context of many studies. If women are scheduled without regard to menstrual cycle phase, researchers should collect this information via self-report (e.g., providing participants with calendars to chart their cycle) or hormonal assessment (e.g., kits that assess reproductive hormones to determine cycle phase). This information could then be examined as a covariate or control variable in analyses.

Because oral contraceptive usage has reliably been associated with differences in salivary cortisol responses, researchers may wish to limit their sample to women who are all on or all off oral contraceptives to reduce variability associated with this medication. However, this might not be feasible in the context of some studies, in which case oral contraceptive usage should be assessed and used as a covariate in analyses. There may be some cases in which it would be optimal to only include women on oral contraceptives; if the protocol requires multiple laboratory sessions over several weeks, having women on oral contraceptives would reduce the within-subject variability between sessions that could stem from being in different phases of the menstrual cycle (since women on OCs do not cycle).

It may be less important in studies assessing plasma (versus salivary) cortisol reactivity to exclude women on the basis of menstrual cycle phase and/or oral contraceptive usage, since this parameter has been shown to be less influenced by these states. However, it is still beneficial to assess cycle phase and medication usage as potential covariates. Further, in some protocols it may be useful to assess *both* salivary and plasma cortisol, in order to compare effects on the free and total cortisol fractions.

Pregnancy and lactation

There are substantial changes in levels of HPA hormones during pregnancy; basal levels of CRH, ACTH, and cortisol increase dramatically as pregnancy progresses. Less is known about how HPA reactivity to psychosocial stressors during pregnancy; however, pregnant women have shown reduced HPA responses to physical stressors and CRH challenges (de Weerth & Buitelaar, 2005, for review). Given the dramatic changes in HPA activity during pregnancy, it is best to exclude all pregnant women from cortisol reactivity protocols (unless of course one is specifically examining the HPA axis in pregnancy).

Only a handful of studies have examined the effects of lactation on cortisol reactivity. Several have found no differences in total cortisol reactivity among lactating versus non-lactating women (Altemus, Redwine, Leong, Frye, Porges, & Carter, 2001; Redwine, Altemus, Leong, & Carter, 2001; for review, see Heinrichs, Neumann, & Ehlert, 2002). However, there could be acute effects of breastfeeding on HPA stress responses; one study randomly assigned women to either breast-feed or hold their infant prior to a psychosocial stressor, and those who had just breast-fed showed reduced cortisol reactivity (Heinrichs et al., 2001). Future research is needed to further understand the potential effects of lactation on HPA responses.

Age and menopausal status

The age of participants has been associated with cortisol reactivity. A meta-analysis found that older adults have a stronger cortisol response to "challenge", including both psychosocial and pharmacological stimuli (Otte et al., 2005). Interestingly, this age effect was much greater among women than men; women showed nearly three times as strong age effect compared to men. This could be due to a variety of factors, but many have hypothesized that it could stem from reductions in estradiol among menopausal women. Post-menopausal women have typically shown greater reactivity compared to their pre-menopausal counterparts (e.g., Kudeilka, Buske-Kirschbaum, Hellhammer, & Kirschbaum, 2004; Seeman, Singer, Wilkinson, & McEwen, 2001), although some have found no differences (e.g., Steptoe, Fieldman, Evans, & Perry, 1996; Kudeilka et al., 1999; see Kajantie & Phillips, 2006, for review).

There have been a handful of studies that have assessed the effects of hormone replacement (HRT) therapy on cortisol reactivity to stressors. For example, a 48-hour estradial patch increased cortisol reactivity in men (Kirschbaum et al., 1996) but had no effect on women (Kudielka et al., 1999). Other studies have shown both decreased (Lindheim et al., 1992) and increased (Burleson et al., 1998) reactivity among women on estradial treatments. Some variability in the findings may be due to length of treatment or method of assessment. Additional studies are

needed to further understand the complex associations between age, gender, and hormonal treatment.

In summary, researchers should obtain information regarding women's menopausal status and hormone replacement therapy treatments in the context of cortisol reactivity protocols. Given the mixed findings regarding HRT, it might be best to exclude women on these treatments.

Health behaviors

A variety of health behaviors can influence basal cortisol levels as well as reactivity to acute stressors. This can be very important to consider when determining exclusion criteria and restrictions before the experimental session, and assessing possible covariates, as health behaviors can explain variability in reactivity and/or lead to difficulties in accurately interpreting the results of investigations.

Smoking can have a significant impact on HPA activity. Smoking a cigarette (independent of a stressor) can acutely increase cortisol levels (Kirschbaum, Wust, Strasburger, 1992). However, smoking has consistently been shown to lead to blunted cortisol responses (for review, see Rohleder & Kirschbaum, 2006). For example, habitual smokers have reduced salivary cortisol reactivity to the TSST compared to non-smokers (Kirschbaum, Scherer, Strasburger 1994), and smoking 30 minutes prior to a stressor has also been associated with blunted cortisol responses (Tsuda, Steptoe, West, Fieldman & Kirschbaum, 1996). Al'Absi and colleagues (al'Absi, Wittmers, Erickson, Hatsukami, & Crouse, 2003) compared non-smokers and smokers who had been randomly assigned to either abstain from smoking since the previous evening or to smoke at their normal rates. They found that both groups of smokers showed decreases in salivary cortisol in response to an acute stressor, whereas non-smokers showed the expected increases. The similarity between the two smoker groups suggests that observed differences between smokers and non-smokers cannot be attributed to the acute effects of smoking.

This has clear methodological implications. As habitual smoking can have consistent effects on cortisol reactivity, it is optimal to exclude smokers from these studies. If smokers are included, then having them refrain from smoking immediately prior to the session (to eliminate the acute effects of smoking on HPA parameters), and collecting information regarding smoking activity (e.g., frequency) for use as a covariate could reduce variability in reactivity attributable to this behavior.

Physical activity can have an effect on the HPA axis. First, acute bouts of exercise can independently increase cortisol levels (e.g., DeRijk et al., 1997; Kirschbaum et al., 1992); however, it appears that exercise must be of moderate intensity and of a prolonged duration to activate the HPA axis. Other studies have examined how habitual exercise or fitness is associated with cortisol reactivity to acute stressors. Most have not found differences in cortisol responses between

participants of varying fitness levels (e.g., Long, 1991; Moyna, Bodnar, Goldberg, Shurin, Robertson, & Rabin, 1999; Sothmann, Gustafson, Garthwaite, Horn, & Hart, 1988); however, many of these studies have failed to elicit cortisol responses to the stressor, and so it is difficult to tell whether fitness level would moderate reactivity using a stressor that activated this system. Differences in reactivity may also only emerge among those very highly-trained; in one study, elite athletes showed reduced cortisol reactivity to the TSST relative to amateur athletes and non-athletes (Rimmele, Seiler, Marti, Wirtz, Ehlert, & Heinrichs, 2009).

Obesity levels have been associated with cortisol reactivity. Several studies have found greater cortisol responses among individuals with higher waist-to-hip ratios (WHR: a marker of central adiposity; Epel et al., 1999; 2000; Moyer, Rodin, Grilo, Cummings, Larson, & Rebuffe-Scrive, 1994). Interestingly, the different WHR groups had similar body mass index scores, and BMI did not predict cortisol reactivity; this suggests that WHR may be more relevant to assess in the context of HPA research.

Taken together, these findings have several methodological implications. First, since acute exercise can stimulate the HPA axis, researchers should restrict (or at least assess) exercise among participants on the day of the experimental session. Additionally, WHR and/or BMI should be assessed or recorded, and examined as a potential covariate in analyses.

Many factors related to diet have been shown to influence cortisol reactivity, including drinking caffeine or alcohol and eating meals. Ingesting caffeine can elevate cortisol levels, independent of a stressor (e.g., al'Absi, Lovallo, McKey, & Pincomb, 1998), and has also been shown to be linked with cortisol reactivity. Ingesting caffeine 45–60 minutes prior to an acute stressor task increases cortisol responses (e.g., al'Absi et al., 1998; Lane, Adcock, Williams, & Kuhn, 1990). Similar effects are seen among habitual and light caffeine drinkers, as there were no interactions between typical caffeine consumption and cortisol reactivity (Lane et al., 1990). Therefore, researchers may wish to restrict participants from drinking caffeinated beverages prior to the experimental session, particularly in the hour beforehand. As alcohol can also elevate cortisol levels (Cobb & van Theil, 1982), it is also best to have participants refrain from drinking alcohol the day of the experimental session. Eating a meal can have a direct, elevating effect on cortisol levels (e.g., Holl, Fehm, Voigt, & Teller, 1984), therefore food consumption should be restricted within at least an hour of a laboratory session.

While eating can lead to acute cortisol elevations, fasting can impact cortisol reactivity. Kirschbaum and colleagues (1997) found that participants who had fasted for 8–11 hours prior to undergoing the TSST did not show significant increases in cortisol. In contrast, those who fasted but then ingested glucose 1 hour prior to the stressor showed robust cortisol responses. A subsequent study (Gonzalez-Bono, Rohleder, Hellhammer, Salvador, & Kirschbaum, 2002) had participants ingest glucose, fat, protein or water 1 hour prior to the TSST. The

glucose load condition showed significantly greater cortisol reactivity compared to the others. Taken together, this indicates that low glucose levels (e.g., fasting) may attenuate cortisol responses, whereas high glucose levels may heighten reactivity. Additionally, it suggests researchers may wish to collect information on the content and timing of meals during the day of an experimental session. If salivary cortisol is assessed, it also is useful to restrict consumption of chips or other foods that could cause micro-tears and/or bleeding of the gums, as this could contaminate the saliva sample.

Sleep is an important parameter that is tied to HPA activity (for review, see Meerlo, Sgoifo, & Suchecki, 2008). The circadian rhythm of cortisol is tied to the sleep–wake cycle, and thus, sleep can have an important effect on diurnal patterns of HPA activity. Several studies have shown cortisol secretion at night is suppressed by sleep (e.g., Vgontzas, Mastorakos, Bixler, Kales, Gold, & Chrousos, 1999; Weibel, Follenius, Spiegel, Ehrhart, & Brandenberger, 1995), and some studies have found that sleep deprivation (e.g., Leproult, Copinschi, Buxton, & Van Cauter, 1997; Spiegel, Leproult, & Van Cauter, 1999) or insomnia (e.g., Vgontzas et al., 2001) is associated with elevations in cortisol levels. In the context of cortisol reactivity studies, assessment of typical sleep patterns (e.g., through standardized questionnaires like the Pittsburgh Sleep Quality Index; Buysse, Reynolds, Monk, Berman, & Kupfer, 1989) and time of awakening on the day of the experimental session could be used as potential covariates in analyses to account for some of the effects of sleep on the HPA axis.

Physical and psychological health conditions

Whether physical and psychological health conditions are associated with dysregulations in or different patterns of cortisol reactivity has been the focus of a tremendous amount of research. These studies have been important for examining how pathophysiological states may influence the functioning and reactivity of the HPA hormones. These studies also have implications for the design of stress reactivity protocols; specifically, with regard to establishing exclusion/inclusion criteria and/or assessing potential covariates.

Generally, restricting cortisol reactivity studies to "healthy" samples without chronic health conditions is the best way to ensure the integrity and interpretability of the data. It also increases the probability that individual differences and/or condition effects are due to underlying psychological processes rather than confounded by health conditions. However, exactly what makes a "healthy" sample is a question of debate. Whether to restrict the sample, and if so, how much, is an important factor to consider.

At the very least, it is wise to exclude individuals with diseases that specifically implicate HPA axis functioning. For example, Addison's disease (where cortisol levels are abnormally low) or Cushing's disease (where cortisol levels are abnormally high)

could have clear effects on the HPA axis, and individuals with these conditions should be excluded from experimental protocols. Other chronic and/or serious health conditions, including depression (e.g., Heim & Nemeroff, 1999), cardiovascular disease (e.g., al'Absi et al., 1998), inflammatory disorders (e.g., asthma, atopic dermatitis, rheumatoid arthritis; Buske-Kirschbaum et al., 1997; Capellino, & Straub, 2008; Priftis, Papadimitriou, Nicolaidou & Chrousos, 2009), metabolic disorders (e.g., diabetes, metabolic syndrome; Bjorntorp, 2001; Kemmer et al., 1986), cancer (e.g., Sephton et al., 2000; van der Pompe, Antoni, & Heijnen, 1996), and PTSD (e.g., Yehuda, 2006) have been associated with dysregulations in HPA activity and/or responses to psychosocial stressors. If participants are not excluded on the basis of these conditions, a comprehensive self-report assessment of chronic health conditions should be utilized for post-hoc exclusion and/or covariate analyses.

Additionally, the use of a variety of prescription and over the counter medications can also influence basal HPA parameters or reactivity to challenge. This is particularly true of corticosteroids and other steroid-based medications. This type of medication usage should be an exclusion criterion and/or assessed by self-report during the experimental session (for either post-hoc exclusion and/or covariate analyses). Acute illness (e.g., flu) can also influence HPA parameters, so it is optimal to re-schedule sessions if a participant is sick.

Comprehensive health/medication restrictions may not exclude too many participants in certain samples (e.g., undergraduate populations); however, it could dramatically limit those who would be eligible to participate among others (e.g., elderly adults). It also could raise questions of the representativeness of a sample; for example, how typical is a group of older adults without health problems and/or on medications? The balance between representativeness of the sample and interpretability of the results must be taken into account and carefully weighed when making decisions about exclusion criteria.

Assays and Analyses

Once a study is complete, samples can be sent to a lab for quantification of cortisol levels. If one does not have the equipment and/or experience to run the assays "in house", there are a number of ways to have samples assayed. Many universities have research labs with the facilities and equipment to run this type of assay; establishing collaborations with endocrinologists, immunologists, or physicians at one's home university or nearby institution may be one method of acquiring the equipment and technical experience necessary to run cortisol assays. The expertise of these collaborators can also be extremely helpful at all stages of the research process. There are also laboratories and/or companies that will process and analyze samples (fees usually are determined at a cost per sample[3]); samples

can be shipped (via a standard delivery service) to the lab of choice for analysis. Ideally, one should determine the laboratory and/or method to assay the cortisol prior to starting the study, as different laboratories have different protocols for labeling, storing, and shipping samples.

After the assays are complete and the data are obtained, it is important to go through a careful data screening process. There can be a number of reasons for outliers with cortisol data; it is often helpful to cross-check outliers with the self-reported health behaviors and health conditions assessed during the experimental session. Sometimes, the reason for outlying values can be identified in this manner (i.e., high values attributable to consumption of a large amount of caffeine prior to the experimental session, use of a specific medication, etc.). As with other types of social/biobehavioral research, data screening and establishing criteria for excluding outliers should follow standard convention (e.g., Tabachnick & Fidell, 2000). Additionally, cortisol values are often not normally distributed; log-transformations may be necessary to fulfill the assumptions of normality required for many statistical analyses.

Many types of data analytic strategies have been used in cortisol reactivity research[4]. Repeated-measures analyses are often conducted in order to capitalize on the multiple time points in which cortisol was assessed; these analyses can be appropriate for establishing whether HPA parameters change over time, and further, depending on the study aims and design, whether there are differences in reactivity among different conditions or different groups of individuals. Multilevel modeling approaches can also be useful when testing if a continuous predictor (e.g., emotion, personality factor, etc.) is associated with cortisol trajectories over time.

Covariates are important to consider in the context of cortisol research. Depending on the exclusion criteria and behavioral restrictions employed, it can be important to establish whether health conditions (e.g., depression, arthritis), medication usage (e.g., oral contraceptives), or health behaviors (sleep, smoking) are associated with baseline cortisol or patterns of reactivity. However, the number of covariates that can be employed is dependent on sample size (Babyack, 2004), and care must be taken not to over-fit the statistical models.

Conclusion

Inclusion of HPA parameters, such as cortisol, in social psychological research represents an opportunity to further understand the physiological correlates of fundamental processes and to delineate the potential health implications of certain social psychological phenomena. However, using the proper methodological procedures and tools to guide the design, interpretation, and dissemination of cortisol reactivity studies is necessary for this growing area of social psychological research.

Notes

1 Please note that salivettes can be purchased with or without citric acid preparation. The citric acid preparation can interfere with certain immunoassays, producing artificially high cortisol values. One should contact the lab that will be processing the samples prior to purchase to ensure the appropriate salivette selection.
2 Excellent information regarding the storage and processing of salivary cortisol can be found at Clemens Kirschbaum's website (http://biopsychologie.tu-dresden.de/eng/5/page_5_0_0_0.php).
3 These include Clemens Kirschbaum's laboratory in Dresden, Germany (http://biopsychologie.tu-dresden.de/eng/5/page_5_0_0_0.php) and Salimetrics, run by Doug Granger (www.salimetrics.com).
4 The "Statistical Corner" in the journal *Psychosomatic Medicine* is an excellent resource, which features user-friendly articles with cutting-edge statistical information and recommendations on analyses and techniques for cortisol and biobehavioral research.

References

Abplanalp, J. M., Livingston, L., Rose, R. M., & Sandwisch, D. (1977). Cortisol and growth hormone responses to psychological stress during the menstrual cycle. *Psychosomatic Medicine, 39*, 158–177.

Adolphs, R. (2002). Trust in the brain. *Nature Neuroscience, 5*, 192–193.

al'Absi, M., Bongard, S., Buchanan, T., Pincomb, G. A., Licinio, J., & Lovallo, W. R. (1997). Cardiovascular and neuroendocrine adjustment to public speaking and mental arithmetic stressors. *Psychophysiology, 34*, 266–275.

al'Absi, M., Lovallo, W. R., McKey, B. S., & Pincomb, G. A. (1998). Hypothalamic-pituitary-adrenocortical responses to psychological stress and caffeine in men at high and low risk for hypertension. *Psychosomatic Medicine, 60*, 521–527.

al'Absi, M., Wittmers, L. E., Erickson, J., Hatsukami, D., & Crouse, B. (2003). Attenuated adrenocorticoal and blood pressure responses to psychological stress in ad libitum and abstinent smokers. *Pharmacology, Biochemistry & Behavior, 74*, 4401–410.

Altemus, M., Redwine, L. S., Leong, Y. M., Frye, C.A., Porges, S. W., & Carter, C. S. (2001b). Responses to laboratory psychosocial stress in postpartum women. *Psychosomatic Medicine, 63*, 814–821.

Amodio, D. M. (2009). Intergroup anxiety effects on the control of racial stereotypes: A psychoneuroendocrine analysis. *Journal of Experimental Social Psychology, 45*, 60–67.

Amodio, D. M., Harmon-Jones, E., & Devine, P. G. (2003). Individual differences in the activation and control of affective race bias as assessed by startle eyeblink response and self-report. *Journal of Personality and Social Psychology, 84*, 738–753.

Andrews, J., Wadiwalla, M., Juster, R. P., Lord, C., Lupien, S. J., & Pruessner, J. C. (2007). Effects of manipulating the amount of social-evaluative threat on the cortisol stress response in young healthy men. *Behavioral Neuroscience, 121*(5), 871–876.

Anthony, B. J. (1985). In the blink of an eye: Implications for reflex modification for information processing. *Advances in Psychophysiology, 1*, 167–218.

Anthony, B., & Graham, F. K. (1983). Evidence for sensory-selective set in young infants. *Science*, *220*, 742–743.

Babyack, M. A. (2004). What you see is may not be what you get: A brief, nontechnical introduction to overfitting in regression-type models. *Psychosomatic Medicine, 66*, 411–421.

Bakker, F. C., Boschker, M. S. J., & Chung, T. (1996). Changes in muscular activity while imagining weight lifting using stimulus or response propositions. *Journal of Sport and Exercise Psychology, 18*, 313–324.

Basmajian, J. V., & De Luca, C. J. (1985). *Muscles alive: Their functions revealed by electromyography* (5th ed.). Baltimore: Williams & Wilkins.

Baumeister, R. F., & Leary, M. R. (1995). The need to belong: Desire for interpersonal attachments as a fundamental human motivation. *Psychological Bulletin, 117*, 497–529.

Bechara, A., Damasio, H., Tranel, D., & Damasio, A. (1997). Deciding advantageously before knowing the advantageous strategy. *Science, 275,* 1293–1295.

Bell, C. (1844). *The anatomy and philosophy of expression as connected with the fine arts* (3th ed.). London: John Murray.

Benning, S. D., Patrick, C. J., & Lang, A. R. (2004). Emotional modulation of the post-auricular reflex. *Psychophysiology, 41*, 426–432.

Berger, S. M., & Hadley, S. W. (1975). Some effects of a model's performance on an observer's electromyographic activity. *American Journal of Psychology, 88*(2), 263–276.

Berntson, G.G., Bigger, J.T. Jr., & Eckberg, D.L. (1997). Heart rate variability: Origins, methods, and interpretive caveats. *Psychophysiology, 34*(6), 623–648.

Berntson, G.G., Cacioppo, J.T., Quigley, K.S. (1993). Cardiac psychophysiology and autonomic space in humans: Empirical perspectives and conceptual implications. *Psychological Bulletin, 114*(2), 296–322.

Bjorntorp, P. (2001). Do stress reactions cause abdominal obesity and comorbidities? *Obesity Review, 2*, 73–86.

Blacher, J., Staessen, J.A., Girerd, X., Gasowske, J., Thijs, L., Liu, L., Wang, J. G., Fagard, R. H., & Safar, M.E. (2000). Pulse pressure not mean pressure determines cardiovascular risk in older hypertensive patients. *Archives of Internal Medicine, 160*, 1085–1089.

Blascovich, J. (2000). Psychophysiological methods. In H. T. Reis, & C. M. Judd (Eds.), *Handbook of research methods in social and personality psychology* (pp. 117–137). Cambridge, UK: Cambridge University Press.

Blascovich, J. (2008). Challenge and threat. In A. J. Elliot (Ed.), *Handbook of approach and avoidance motivation* (pp. 431–446). New York: Erlbaum.

Blascovich, J., & Ginsburg, G.P. (1978). Conceptual analysis of risk taking in 'risky shift' research. *Journal for the Theory of Social Behavior, 8*, 217–230.

Blascovich, J., & Katkin, E. S. (1993). Psychological stress testing for coronary heart disease. In J. Blascovich, & E. S. Katkin (Eds.), *Cardiovascular reactivity to psychological stress and disease* (pp. 27–48) Washington, D. C.: American Psychological Association.

Blascovich, J., & Mendes, W. B. (2000). Challenge and threat appraisals: The role of affective cues. In J. Forgas (Ed.), *Feeling and thinking: The role of affect in social cognition* (pp. 59–82). Cambridge, UK: Cambridge University Press.

Blascovich, J., & Mendes, W. B. (2010). Social psychophysiology and embodiment. In D. Gilbert, S., Fiske, & G. Lindzey, (Eds.), *Handbook of Social Psychology* (5th ed., pp. 194–227). New York: Wiley.

Blascovich, J., Mendes, W. B., Hunter, S.B. Lickel, B., & Kowai-Bell, N. (2001). Perceiver threat in social interactions with stigmatized others. *Journal of Personality and Social Psychology, 80*, 253–267.

Blascovich, J., Mendes, W.B., & Seery, M. (2002). Intergroup encounters and threat: A multi-method approach. In D. Mackie, & E. Smith (Eds.), *From prejudice to intergroup emotions: Differentiated reactions to social groups* (pp. 89–110). New York: Psychology Press.

Blascovich, J., Mendes, W. B., Tomaka, J., Salomon, K., & Seery, M. D. (2003). The robust nature of the biopsychosocial model of challenge and threat: A reply to Wright and Kirby. *Personality and Social Psychology Review, 7*(3), 234–243.

Blascovich, J., & Seery, M.D. (2007). Visceral and somatic indexes of social psychological constructs. In A. Kruglanski & E.T. Higgins (Eds.). *Social psychology: Handbook of basic principles Second Edition*. (pp. 19 – 38). New York: Guilford.

Blascovich, J., Seery, M., Mugridge, C., Weisbuch, M., & Norris, K. (2004). Predicting athletic performance from cardiovascular indicators of challenge and threat. *Journal of Experimental Social Psychology, 40,* 683–688.

Blascovich, J., & Tomaka, J. (1996). The biopsychosocial model of arousal regulation. *Advances in experimental social psychology, 28*, 1–51.

Blumenthal, T. D., Cuthbert, B. N., Filion, D. L., Hackley, S., Lipp, O. V., & van Boxtel, A. (2005). Committee report: Guidelines for human startle eyeblink electromyographic studies. *Psychophysiology, 42*, 1–15.

Blumenthal, T. D., Elden, A., & Flaten, M. A. (2004). A comparison of several methods used to quantify prepulse inhibition of eyeblink responding. *Psychophysiology, 41*, 326–332.

Blumenthal, T. D., & Franklin, J. C. (2009). The startle eyeblink response. In E. Harmon-Jones & J. S. Beer (Eds.), *Methods in social neuroscience* (pp. 92–117). New York: Guilford Press.

Bohlin, G., Eliasson, K., Hjemdahl, P., Klein, K., & Frankenhaeuser, M. (1986). Pace variation and control of work pace as related to cardiovascular, neuroendocrine, and subjective responses. *Biological Psychology, 23*, 247–263.

Bosch, J. A., de Geus, E. J., Carroll, D., Goedhart, A. D., Anane, L. A., van Zanten, J.J., Helmerhost, E. J., & Edwards, K. M. (2009). Social-evaluative threat determines the magnitude but not the pattern of psychological responses. *Psychosomatic Medicine, 71*(8), 877–85.

Bradley, M. M., Cuthbert, B. N., & Lang, P. J. (1993). Pictures as prepulses: Attention and emotion in startle modification. *Psychophysiology, 30*, 541–545.

Bradley, M. M., & Lang, P. J. (2007). Emotion and motivation. In J. T. Cacioppo, L. G. Tassinary, & G. G. Berntson (Eds.), *Handbook of psychophysiology* (3rd ed., pp. 581–607). Cambridge, UK: Cambridge University Press.

Bramsley, G. R., Bruun, G., Buchthal, F., Guld, C., & Petersen, H. S. (1967). Reduction of electrical interference in measurements of bioelectric potentials in a hospital. *Acta Polytechnica Scandanavia Electric Engineering Series, 15*, 1–37.

Breier, A. (1989). Experimental approaches to human stress research: Assessment of neurobiological mechanisms of stress in volunteers and psychiatric patients. *Biological Psychiatry, 26*, 438–462.

Brinkworth, R. S. A., & Turker, K. S. (2003). A method for quantifying reflex responses from intra-muscular and surface electromyogram. *Journal of Neuroscience Methods, 122*, 179–193.

Britt, T. W., & Blumenthal, T. D. (1992). The effects of anxiety on motoric expression of the startle response. *Personality and Individual Differences, 13*, 91–97.

Brown, L. M., Bradley, M. M., & Lang, P. J. (2006). Affective reactions to pictures of ingroup and outgroup members. *Biological Psychology, 71*, 303–311.

Brownley, K.A., Hurwitz, B.E., & Schneiderman, N. (2000) Cardiovascular psychophysiology. In J. T. Cacioppo, L. G. Tassinary, and G. G. Berntson, (Eds.), *Handbook of psychophysiology* (2nd ed., pp. 224–264). New York: Cambridge University Press.

Burleson, M. H., Malarkey, W. B., Cacioppo, J. T., Poehlmann, K. M., Kiecolt-Glaser, J. K., Berntson, G. G., & Glaser, R. (1998). Postmenopausal hormone replacement: Effects on

autonomic, neuroendocrine, and immune reactivity to brief psychological stressors. *Psychosomatic Medicine, 60*, 17–25.

Bush, L. K., Hess, U., & Wolford, G. (1993). Transformations for within-subjects designs: A Monte Carlo investigation. *Psychological Bulletin, 113*, 566–579.

Buske-Kirschbaum, A., Jobst, S., Wustmans, A., Kirschbaum, C., Rauh, W., & Hellhammer, D. (1997). Attenuated free cortisol response to psychosocial stress in children with atopic dermatitis. *Psychosomatic Medicine, 59*, 419–426.

Buysse, D. J., Reynolds, C. F., Monk, T. H., Berman, S. R., & Kupfer, D. J. (1989). The Pittsburgh Sleep Quality Index: A new instrument for psychiatric practice and research. *Psychiatry Research, 28*, 193–213.

Cacioppo, J. T., Hawkley, L. C., & Crawford, E. (2002). Loneliness and health: Potential mechanisms. *Psychosomatic Medicine*, *64*(3), 407–417.

Cacioppo, J. T., Marshall-Goodell, B. S., & Dorfman, D. D. (1983). Skeletal muscle patterning: Topographical analysis of the integrated electromyogram. *Psychophysiology, 20*, 269–283.

Cacioppo, J. T., Martzke, J. S., Petty, R. E., & Tassinary, L. G. (1988). Specific forms of facial EMG response index emotions during an interview: From Darwin to the continuous flow hypothesis of affect-laden information processing. *Journal of Personality and Social Psychology, 54*, 592–604.

Cacioppo, J. T., & Petty, R. E. (1979). Attitudes and cognitive response: An electrophysiological approach. *Journal of Personality and Social Psychology, 37*, 2181–2199.

Cacioppo, J. T., & Petty, R. E. (1981). Electromyograms as measures of extent and affectivity of information processing. *American Psychologist, 36*, 441–456.

Cacioppo, J. T., & Petty, R. E. (1983). Foundations of social psychophysiology. In J. T. Cacioppo, & R. E. Petty (Eds.), *Social psychophysiology: A sourcebook* (pp. 3–36). New York: Guilford.

Cacioppo, J. T., Petty, R. E., Losch, M. E., & Kim, H. S. (1986). Electromyographic activity over facial muscle regions can differentiate the valence and intensity of affective reactions. *Journal of Personality and Social Psychology, 50*(2), 260–268.

Cacioppo, J. T., Rourke, P. A., Marshall-Goodell, B. S., Tassinary, L. G., & Baron, R. S. (1990). Rudimentary physiological effects of mere observation. *Psychophysiology, 27*, 177–186.

Cacioppo, J. T., & Tassinary, L. G. (1990). Inferring psychological significance from physiological signals. *American Psychologist, 45,* 16–28.

Cacioppo, J. T., Tassinary, L. G., & Berntson, G. G. (2007) *Handbook of Psychophysiology* (3rd ed.). Cambridge, UK: Cambridge University Press.

Capellino, S., & Straub, R. H. (2008). Neuroendocrine immune pathways in chronic arthritis. *Best Practice & Research Clinical Rheumatology, 22*(2), 285–297.

Cattaneo, L., Fabbri-Destro, M., Boria, S., Pieraccini, C., Monti, A., Cossu, G., & Rizzolatti, G. (2007). Impairment of actions chains in autism and its possible role in intention understanding. *Proceedings of the National Academy of Sciences, 104*(45), 17825–17830.

Chesney, M., & Rosenman, R. H. (Eds.), (1985). *Anger and hostility in Cardiovascular and Behavioral Disorders*. Washington, D. C.: Hemispheric Publishing.

Chobanian, A. V., Bakris, G. L., Black, H. R., Cushman, W. C., Green, A. L., Izzo, J. L. Jr, Jones, D. W., Materson, B. J., Oparil, S., Wright, J. T. Jr, & Roccella, E. J.: the National

High Blood Pressure Education Program Coordinating Committee (2003). Seventh report of the Joint National Committee on prevention, detection, evaluation, and treatment of high blood pressure. *Hypertension, 42*, 1206–1252.

Clow, A., Thorn, L., Evans, P., & Hucklebridge, F. (2004). The awakening cortisol response: Methodological issues and significance. *Stress, 7*, 29–37.

Cobb, C. F., & van Theil, D. H. (1982). Mechanism of ethanol-induced adrenal stimulation. *Alcoholism: Clinical and Experimental Research, 6*(2), 202–206.

Cohen, S., Hamrick, N., Rodriguez, M. S., Feldman, P. J., Rabin, B. S., & Manuck, S. B. (2000). The stability of and intercorrelations among cardiovascular, immune, endocrine, and psychological reactivity. *Annals of Behavioral Medicine, 22*, 171–179.

Cohen, S., Schwartz, J. E., Epel, E., Kirschbaum, C., Sidney, S., & Seeman, T. (2006). Socioeconomic status, race, and diurnal cortisol decline in the Coronary Artery Risk Development in Young Adults (CARDIA) Study. *Psychosomatic Medicine, 68,* 41–50.

Creswell, J. D., Welch, W. T., Taylor, S. E., Sherman, D. K., Gruenewald, T. L., & Mann, T. (2005). Affirmation of personal values buffers neuroendocrine and psychological stress responses. *Psychological Science, 16*, 846–851.

Croizet**,** J., Després, G., & Gauzins, M. (2004). Stereotype threat undermines intellectual performance by triggering a disruptive mental load. *Personality and Social Psychology Bulletin, 30,* 721–731.

Dambrun, M., Despres, G., & Guimond, S. (2004). On the multifaceted nature of prejudice: Psychophysiology responses to ingroup and outgroup ethnic stimuli. *Current Research in Social Psychology, 8*, 187–204.

Dandeneau, S. D., Baldwin, M. W., Baccus, J. R., Sakellaropoulo, M., & Pruessner, J. (2007). Cutting stress off at the pass: Reducing vigilance and responsiveness to social threat by manipulating attention. *Journal of Personality and Social Psychology, 93*, 651–666.

Darwin, C. (1872). *The expression of emotions in man and animals*. New York: Appleton.

Davis, J. F., Malmo, R. B., & Shagass, C. (1954). Electromyographic reaction to strong auditory stimulation in psychiatric patients. *Canadian Journal of Psychology, 8*, 177–186.

Dawson, M. E., Schell, A. M., & Böhmelt, A. H. (Eds.), (1999). *Startle modification: Implications for neuroscience, cognitive science, and clinical science*. Cambridge, UK: Cambridge University Press.

Delisle, A., Lariviere, C., Plamondon, A., & Salazar, E. (2009). Reliability of different thresholds for defining muscular rest of the trapezius muscles in computer office workers. *Ergonomics, 52*(7), 860–871.

Demaree, H. A., & Everhart, D. E. (2004). Healthy high-hostiles: Reduced parasympathetic activity and decreased sympathovagal flexibility during negative emotional processing. *Personality and Individual Differences, 36*(2), 457–469.

Denver, J. W., Reed, S. F., & Porges, S. W. (2007) Methodological issues in the quantification of respiratory sinus arrhythmia. *Biological Psychology, 74*(2), 286–294.

DeRijk, R., Michelson, D., Karp, B., Petrides, J., Galliven, E., Deuster, P., Paciotti, G., Gold, P. W., & Sternberg, E. M. (1997). Exercise and circadian rhythm-induced variations in plasma cortisol differentially regulate interleukin-1 beta (Il-1 beta), Il-6, and tumor necrosis factor-alpha (TNF-alpha) production in humans: High sensitivity of TNF alpha and resistance of Il-6. *Journal of Clinical Endocrinology and Metabolism, 82*(7), 2182–2191.

Dess, N. K., Linwick, D., Patterson, J., Overmier, J. B., & Levine, S. (1983). Immediate and proactive effects of controllability and predictability on plasma cortisol responses to shocks in dogs. *Behavioral Neuroscience, 97*, 1005–16.

de Weerth, C., & Buitelaar, J. K. (2005). Physiolgoical stress reactivity in human pregnancy: A review. *Neuroscience and Biobehavioral Reviews, 29*, 295–312.

Dickerson, S. S., & Kemeny, M. E. (2004). Acute stressors and cortisol responses: A theoretical integration and synthesis of laboratory research. *Psychological Bulletin, 130*(3), 355–391.

Dickerson, S. S., Mycek, P. J., & Zaldivar, F. (2008). Social evaluation – but not mere social presence – elicits cortisol responses to a laboratory stressor task. *Health Psychology, 27*(1), 116–121.

Dienstbier, R. (1989). Arousal and physiological toughness: Implications for mental and physical health. *Psychological Review, 96*, 84–100.

Dorfman, D. D., & Cacioppo, J. T. (1990). Waveform moment analysis: Topographical analysis of nonrhythmic waveforms. In J. T. Cacioppo, & L. G. Tassinary (Eds.), *Principles of psychophysiology* (pp. 661–707). New York: Cambridge University Press.

DuBrul, E. L. (1980). *Sicher's Oral Anatomy*. St. Louis, MO: Mosby.

Duchenne, G. B. (1990). *The mechanisms of human facial expression* (R. A. Cuthbertson, Editor & Trans.). New York: Cambridge University Press. (Original work published 1862).

Eason, R. G., & White, C. T. (1961). Muscular tension, effort, and tracking difficulty: Studies of parameters which affect tension levels and performance efficiency. *Perceptual and Motor Skills, 12*, 331–372.

Ekman, P. (1992). Facial expressions of emotion: New findings, new questions. *Psychological Science, 3*, 34–38.

Ekman, P., & Friesen, W. V. (1978). *The Facial Action Coding System: A technique for the measurement of facial movement*. Palo Alto, CA: Consulting Psychologists Press.

Epel, E., McEwen, B., Seeman, T., Matthews, K., Castellazzo, G., Brownell, K. D., Bell, J., & Ickovics, J. R. (2000). Stress and body shape: Stress-induced cortisol secretion is consistently greater among women with central fat. *Psychosomatic Medicine, 62*, 623–632.

Epel, E., Moyer, A. E., Martin, C. D., Macary, S., Cummings, N., Rodin, J., & Rebuffe-Scrive, M. (1999). Stress-induced cortisol, mood, and fat distribution in men. *Obesity Research, 7*, 9–15.

Fabbri-Destro, M., & Rizzolatti, G. (2008). Mirror neurons and mirror systems in monkeys and humans. *Physiology, 23*, 171–179.

Filion, D. L., Dawson, M. E., & Schell, A. M. (1993). Modification of the acoustic startle-reflex eyeblink: A tool for investigating early and late attentional processes. *Biological Psychology, 35*, 185–200.

Filion, D. L., Dawson, M. E., & Schell, A. M. (1998). The psychological significance of human startle eyeblink modification: A review. *Biological Psychology, 47*, 1–43.

Feinberg, S.E., & Stern, P. (Eds.), (2002). *The polygraph and lie detection.* Washington, D. C.: National Academies Press.

Fridlund, A. J. (1991). Sociality of solitary smiling: Potentiation by an implicit audience. *Journal of Personality and Social Psychology, 60*(2), 229–240.

Fridlund, A. J. (1994). *Human facial expression: An evolutionary view*. San Diego, CA: Academic Press.

Fridlund, A. J., & Cacioppo, J. T. (1986). Guidelines for human electromyographic research. *Psychophysiology, 23*, 567–589.

Fridlund, A. J., Fowler, S. C., & Pritchard, D. A. (1980). Striate muscle tensional patterning in frontalis EMG biofeedback. *Psychophysiology, 17*, 47–55.

Fries, E., Dettenborn, L., & Kirschbaum, C. (2009). The cortisol awakening response (CAR): Facts and future directions. *International Journal of Psychophysiology, 72*, 67–73.

Gold, P. W., Licinio, J., Wong, M., & Chrousos, G. P. (1995). Corticotropin releasing hormone in the pathophysiology of melancholic and atypical depression and in the mechanism of action of antidepressant drugs. *Annals of the New York Academy of Sciences, 771*, 716–729.

Gonzalez-Bono, E., Rohleder, N., Hellhammer, D. H., Salvador, A., & Kirschbaum, C. (2002). Glucose but not protein or fat load amplifies the cortisol response to psychosocial stress. *Hormones and Behavior, 41*, 328–333.

Gramzow, R. H., & Willard, G. B. (2006). Exaggerating current and past performance: Motivated self-enhancement versus reconstructive memory. *Personality and Social Psychology Bulletin, 32*(8), 1114–1125.

Gramzow, R. H., Willard, G. B., & Mendes, W. B. (2008). Big tales and cool heads: Academic exaggeration is related to cardiac vagal reactivity. *Emotion*, 8, 138–144.

Gramzow, R. H., Willard, G. B., & Mendes, W. B. (in press). Big tales and cool heads: GPA exaggeration is related to increased parasympathetic activation. *Emotion.*

Grillon, C., & Davis, M. (1995). Acoustic startle and anticipatory anxiety in humans: Effects of monaural right and left ear stimulation. *Psychophysiology, 32*, 155–161.

Grossman, P., & Taylor, E.W. (2007). Toward understanding respiratory sinus arrhythmia: Relations to cardiac vagal tone, evolution and biobehavioral functions. *Biological Psychology, 74*(2), 263–285.

Gruenewald, T. L., Kemeny, M. E., Aziz, N., & Fahey, J. L. (2004). Acute threat to the social self: Shame, social self-esteem, and cortisol activity. *Psychosomatic Medicine, 66*, 915–924.

Guglielmi, R. S. (1999). Psychophysiological assessment of prejudice: Past research, current status, and future directions. *Personality and Social Psychology Review, 3*(2), 123–157.

Gunnar, M. R., Talge, N. M., & Herrera, A. (2009). Stressor paradigms in developmental studies: What does and does not work to produce mean increases in salivary cortisol. *Psychoneuroendocrinology, 34(7),* 953–967.

Hackley, S. A., & Graham, F. K. (1983). Early selective attention effects on cutaneous and acoustic blink reflexes. *Physiological Psychology, 11*, 235–242.

Hackley, S. A., Muñoz, M. A., Hebert, K., Valle-Inclan, F., & Vila, J. (2009). Reciprocal modulation of eye-blink and pinna-flexion components of startle during reward anticipation. *Psychophysiology, 46*(6), 1154–1159.

Hager, J. C., Ekman, P., & Friesen, W. V. (2002). *Facial action coding system*. Salt Lake City, UT: A Human Face.

Harmon-Jones, E. & Peterson, C. K. (2009). Supine body position reduces neuralresponse to anger evocation. *Psychological Science, 20,* 1209–1210.

Hasbroucq, T., Burle, B., Vidal, F., & Possamaï, C. (2009). Stimulus-hand correspondence and direct response activation: An electromyographic analysis. *Psychophysiology, 46*(6), 1160–1169.

Hawkley, L. C., Burleson, M. H., & Berntson, G. G. (2003). Loneliness in everyday life: Cardiovascular activity, psychosocial context, and health behaviors. *Journal of Personality and Social Psychology*, *85*(1), 105–120.

Hazlett, R. L., & Hazlett, S. Y. (1999). Emotional response to television commercials: Facial EMG versus self-report. *Journal of Advertising Research, 39(2),* 7–23.

Heim, C., Ehlert, U., & Hellhammer, D. H. (2000). The potential role of hypocortisolism in the pathophysiology of stress-related bodily disorders, *Psychoneuroendocrinology, 25*, 1–35.

Heim, C., & Nemeroff, C. B. (1999). The impact of early adverse events experiences on brain systems involved in the pathophysiology of anxiety and affective disorders. *Biological Psychiatry, 46*(11), 1509–22.

Heinrichs, M., Meinlschmidt, G., Neumann, I., Wagner, S., Kirschbaum, C., Ehlert, U., & Hellhammer, D. H. (2001). Effects of suckling on hypothalamic-pituitary-adrenal axis responses to psychosocial stress in postpartum lactating women. *Journal of Clinical Endocrinology & Metabolism, 86,* 4798–4804.

Heinrichs, M., Neuman, I., Ehlert, U. (2002). Lactation and stress: Protective effects of breast-feeding in humans. *Stress, 5,* 195–203.

Hess, U. (2009). Facial EMG. In E. Harmon-Jones & J. S. Beer (Eds.), *Methods in social neuroscience* (pp. 70–91). New York: Guilford Press.

Hess, U., Banse, R., & Kappas, A. (1995). The intensity of facial expression is determined by underlying affective state and social situation. *Journal of Personality and Social Psychology, 69*(2), 280–288.

Hess, U., & Blairy, S. (2001). Facial mimicry and emotional contagion to dynamic emotional facial expressions and their influence on decoding accuracy. *International Journal of Psychophysiology, 40*, 129–141.

Hess, U., Kappas, A., McHugo, G. J., Kleck, R. E., & Lanzetta, J. T. (1989). An analysis of the encoding and decoding of spontaneous and posed smiles: The use of facial electromyography. *Journal of Nonverbal Behavior, 13*(2), 121–137.

Hess, U., Sabourin, G., & Kleck, R. E. (2007). Postauricular and eyeblink startle responses to facial expressions. *Psychophysiology, 44*, 431–435.

Hess, U., & Thibault, P. (2009). Darwin and emotional expression. *American Psychologist, 64*(2), 120–128.

Het, S., Rohleder, N., Schoofs, D., Kirschbaum, C., & Wolf, O. T. (2009). Neuroendocrine and psychometric evaluation of a placebo version of the "Trier Social Stress Test". *Psychoneuronendocrinology, 34*(7), 1075–1086.

Holl, R., Fehm, H. L., Voigt, K. H., & Teller, W. (1984). The 'midday surge' in plasma cortisol induced by mental stress. *Hormone and Metabolic Research, 16*, 158–159.

Izard, C. E. (1979). *The maximally discriminative facial movement coding system (MAX)*. Newark, DE: Psychology Department, University of Delaware.

Jansen, L. M. C., Wied, C. C. G., & Kahn, R. S. (2000). Selective impairment in the stress response in schizophrenic patients. *Psychopharmacology, 149*, 319–325

Jennings, J. R., & Gianaros, P. J. (2007). Methodology. In J. T. Cacioppo, L. G. Tassinary, & G. G. Berntson (Eds.), *Handbook of psychophysiology* (3rd ed., pp. 812–833). Cambridge, UK: Cambridge University Press.

Jennings, P. D., Schell, A. M., Filion, D. L., & Dawson, M. E. (1996). Tracking early and late stages of information processing: Contributions of startle eyeblink reflex modification. *Psychophysiology, 33*, 148–155.

Jones, E. E., & Sigall, H. (1971). The bogus pipeline: A new paradigm for measuring affect and attitude. *Psychological Bulletin, 76*, 349–364.

Kajantie, E. & Phillilps, D. I. (2006). The effects of sex and hormonal status on the physiological response to acute psychosocial stress. *Psychoneuroendocrinology, 31*, 151–178.

Kappas, A. (2003). What facial activity can and cannot tell us about emotions. In M. Katsikitis (Ed.), *The human face: Measurement and meaning* (pp. 215–234). Dordrecht, Netherlands: Kluwer Academic Publishers.

Kassam, K., Koslov, K., & Mendes, W. B. (2009). Decisions under distress: Stress profiles influence anchoring and adjustment. *Psychological Science, 20,* 1394–1399.

Keeter, S. & Samaranayake, N. (February 7, 2007). Can You Trust What Polls Say about Obama's Electoral Prospects? Pew Research Center. http://www.bluedogs.us/can_you_trust_what_polls_say_about_obama%27s_electoral_prospects.htm

Kelly, O., Matheson, M., Martinez, A., Merali, Z., & Anisman, H. (2007). Psychosocial stress evoked by a virtual audience: Relation to neuroendocrine activity. *CyberPsychology & Behavior, 10*(5), 655–662.

Kemmer, F. W., Bisping, R., Steingruber, H. J., Baar, H., Hardtmann, F., Schlaghecke, R., & Berger, M. (1986). Psychological stress and metabolic control in patients with type I diabetes mellitus. *New England Journal of Medicine, 314*, 1078–1084.

Kiecolt-Glaser, J. K., Glaser, R., Cacioppo, J. T., MacCallum, R. C., Snydersmith, M., Kim, C., & Malarkey, W. B. (1997). Marital conflict in older adults: Endocrinological and immunological correlates. *Psychosomatic Medicine, 59*, 339–349.

Kirschbaum, C., Gonzalez-Bono, E., Rohleder, N., Gessner, C., Pirke, K. M., Salvador, A., Hellhammer, D. H. (1997). Effects of fasting and glucose load on free cortisol responses to stress and nicotine. *Journal of Clinical Endocrinology & Metabolism, 82*, 1101–1105.

Kirschbaum, C. & Hellhammer, D. H. (1989). Salivary cortisol in psychobiological research: An overview. *Neuropsychobiology, 22*(3), 150–169.

Kirschbaum, C. & Hellhammer, D. H. (1994). Salivary cortisol in psychoneuroendocrine research: Recent developments and applications. *Psychoneuroendocrinology, 19*, 313–333.

Kirschbaum, C., Kudielka, B. M., Gaab, J., Schommer, N. C., & Hellhammer, D. H. (1999). Impact of gender, menstrual cycle phase, and oral contraceptives on the activity of the hypothalamus-pituitary-adrenal axis. *Psychosomatic Medicine, 61*, 154–162.

Kirschbaum, C., Pirke, K. M., & Hellhammer, D. H. (1993). The 'Trier Social Stress Test': A tool for investigating psychobiological responses in a laboratory setting. *Neuropsychobiology, 28*, 76–81.

Kirschbaum, C., Pirke, K. M., & Hellhammer, D. H. (1995b). Preliminary evidence for reduced cortisol responsivity to psychological stress in women using oral contraceptive medication. *Psychoneuroendocrinology, 20*, 509–514.

Kirschbaum, C., Prussner, J. C., Stone, A. A., Federenko, I., Gaab, J., Lintz, D., Schommer, N., & Hellhammer, D. H. (1995a). Persistent high cortisol responses to repeated psychological stress in a subpopulation of healthy men. *Psychosomatic Medicine, 57*, 468–474.

Kirschbaum, C., Scherer, G., Strasburger, C. J. (1994). Pituitary and adrenal hormone responses to pharmacological, physical, and psychological stimulation in habitual smokers and nonsmokers. *Clinical Investigation, 72*, 804–810.

Kirschbaum , C., Schommer, N., Federenko, I., Gaab, J., Neumann, O., Oellers, M., Rohleder, N., Untiedt, A., Hanker, J., Pirke, K. M., and Hellhammer, D. H. (1996). Short-term estradiol treatment enhances pituitary-adrenal axis and sympathetic responses to spychosocial stress in healthy young men. *Journal of Clinical Endocrinology and Metabolism, 81*, 3639–3643.

Kirschbaum, C., Wust, S., & Hellhammer, D. (1992). Consistent sex differences in cortisol responses to psychological stress. *Psychosomatic Medicine, 54*, 648–657.

Kirschbaum, C., Wust, S., & Strasburger, C. J. (1992). Normal cigarette smoking increase free cortisol in habitual smokers. *Life Science, 50*, 435–442.

Krumhuber, E., Manstead, A. S. R., Cosker, D., Marshall, D., Rosin, P. L., & Kappas, A. (2007). Facial dynamics as indicators of trustworthiness and cooperative behavior. *Emotion, 7*(4), 730–735.

Kudeilka, B. M., Buske-Kirschbaum, A., Hellhammer, D. H., & Kirschbaum, C. (2004). HPA axis response to laboratory psychosocial stress in healthy elderly adults, younger adults, and children: Impact of age and gender. *Psychoneuroendocrinology, 29*, 83–98.

Kudeilka, B. M., Hellhammer, J., Hellhammer, D. H., Wolf, O. T., Varadi, E., Pilz, E., & Kirschbaum, C. (1999). Psychological and endocrine responses to psychosocial stress and Dex-CRF in healthy postmenopausal women and young controls: The impact of age and two-week estradiol treatment. *Neurodendocrinology, 70*, 422–430.

Kudielka, B. M., & Kirschbaum, C. (2005). Sex differences in HPA axis responses to stress: A review. *Biological Psychology, 69*, 113–132.

Kudielka, B. M., Schommer, N. C., Hellhammer, D. H., & Kirschbaum, C. (2004). Acute HPA axis responses, heart rate, and mood changes to psychosocial stress (TSST) in humans at different times of the day. *Psychoenuroendocrinology, 29*, 983–992.

Lane, J. D., Adcock, R. A., Williams, R. B., & Kuhn, C. M. (1990). Caffeine effects on cardiovascular and neuroendocrine responses to acute psychosocial stress and their relationship to level of habitual caffeine consumption. *Psychosomatic Medicine, 52*, 320–336.

Lang, P. J., Bradley, M. M., & Cuthbert, B. N. (1990). Emotion, attention, and the startle reflex. *Psychological Review, 97*, 377–398.

Larsen, J. T., & Norris, J. I. (2009). A facial electromyographic investigation of affective contrast. *Psychophysiology, 46*, 831–842.

Lee, Y., Lopez, D. E., Meloni, E. G., & Davis, M. (1996). A primary acoustic startle pathway: Obligatory role of cochlear root neurons and the reticularis pontis caudalis. *Journal of Neuroscience, 16*, 3775–3789.

Lerner, J. S., Dahl, R. E., Hariri, A. R., & Taylor, S. E. (2007). Facial expressions of emotion reveal neuroendocrine and cardiovascular stress responses. *Biological Psychiatry, 61(2),* 253–260.

Leproult, R., Copinschi, G., Buxton, O., & Van Cauter, E. (1997). Sleep loss results in an elevation of cortisol levels the next evening. *Sleep, 20*, 865–870.

Levine, S. & Ursin, H. (1991). What is stress? In M. R. Brown, G. F. Koob, & C. Rivier (Eds.), *Stress, neurobiology and neuroendocrinology* (pp. 3–21). New York: Dekker.

Linden, W., Earle, T. L., Gerin, W., & Christenfeld, N. (1997). Physiological stress reactivity and recovery: Conceptual siblings separated at birth? *Journal of Psychosomatic Research, 42*, 117–135.

Lindheim, S. R., Legro, R. S., Bernstein, L., Stanczyk, F. Z., Vijod, M. A., Presser, S. C., & Lobo, R. A. (1992). Behavioral stress responses in premenopausal and postmenopausal women and the effects of estrogen. *American Journal of Obstetrics and Gynecology, 167*, 1831–1836.

Lipp, O. V., Siddle, D. A. T., & Dall, P. J. (1997). The effect of emotional and attentional processes on blink startle modification and on electrodermal responses. *Psychophysiology, 34*, 340–347.

Lippold, O. C. J. (1967). Electromyography. In P. H. Venables, & I. Martin (Eds.), *Manual of psychophysiological methods* (pp. 249–298). New York: Wiley.

Long, B. C. (1991). Physiological and psychological stress recovery of physically fit and unfit women. *Canadian Journal of Behavioural Science, 23*, 53–65.

Lovallo, W. R., & Thomas, T. L. (2000). Stress hormones in psychophysiological research: Emotional, behavioral and cognitive implications. In J. T. Cacioppo, L. G. Tassinary, & G. G. Berntson (Eds.), *Handbook of Psychophysiology* (pp. 342–367). Cambridge, UK: Cambridge University Press.

Lupien, S. J., Gaudreau, S., Tchiteya, B. M., Maheu, F., Sharma, S., Nair, N. P., Hauger, R. L., McEwen, B. S., & Meaney, M. J. (1997). Stress-induced declarative memory impairment in healthy elderly subjects: Relationship to cortisol reactivity. *Journal of Clinical Endocrinology and Metabolism, 82*, 2070–2075.

Malarkey, W. B., Kiecolt-Glaser, J. K., Pearl, D., & Glaser, R. (1994). Hostile behavior during marital conflict alters pituitary and adrenal hormones. *Psychosomatic Medicine, 56*, 41–51.

Marshall-Goodell, B. S., Tassinary, L. G., & Cacioppo, J. T. (1990). Principles of bioelectrical measurement. In J. T. Cacioppo & L. G. Tassinary (Eds.), *Principles of psychophysiology: Physical, social, and inferential elements* (pp. 113–148). New York: Cambridge University Press.

Mason, J. W. (1968). A review of psychoendocrine research on the pituitary-adrenal cortical system. *Psychosomatic Medicine, 30*, 576–607.

Matthews, K. A. (1988). Coronary heart disease and Type A behaviors: Update on and alternative to the Booth-Kewley and Friedman (1987) quantitative review. *Psychological Bulletin, 104,* 373–380.

McEwen, B. S. (1998). Protective and damaging effects of stress mediators. *New England Journal of Medicine, 338*, 171–179.

McEwen, B. S. (2004). Protection and damage from acute and chronic stress: Allostasis and allostatic overload and relevance to the pathophysiology of psychiatric disorders. *Annals of the New York Academy of Sciences, 1032*, 1–7.

McGarry, T., & Franks, I. (1997). A horse race between independent processes: Evidence for a phantom point of no return in the preparation of a speeded motor response. *Journal of Experimental Psychology: Human Perception and Performance, 23*, 1533–1542.

McGuigan, F. J. (1978). *Cognitive psychophysiology: Principles of covert behavior*. Englewood Cliffs, NJ: Prentice-Hall.

McGuigan, F. J., & Bailey, S. C. (1969). Longitudinal study of covert oral behavior during silent reading. *Perceptual and Motor Skills, 28*, 170.

McHugo, G. J., & Lanzetta, J. T. (1983). Methodological decision in social psychophysiology. In J. T. Cacioppo, & R. E. Petty (Eds.), *Social psychophysiology: A sourcebook* (pp. 630–665). New York: Guilford Press.

Meerlo, P., Sgoifo, A., & Suchecki, D. (2008). Restricted and disrupted sleep: Effects on autonomic function, neuroendocrine stress systems and stress responsivity. *Sleep Medicine Reviews, 12*, 197–210.

Mendes, W. B., & Barrett, L. F. (in preparation). *Body position blunts emotional responding*.

Mendes, W. B., Blascovich, J., Hunter, S. B., Lickel, B., & Jost, J. T. (2007). Threatened by the unexpected: Physiological responses during social interactions with expectancy-violating partners. *Journal of Personality and Social Psychology, 92*, 698–716.

Mendes, W. B., Blascovich, J., Lickel, B., & Hunter, S. (2002). Challenge and threat during interactions with White and Black men. *Personality and Social Psychology Bulletin, 28*, 939–952.

Mendes, W. B., Gray, H. M., Mendoza-Denton, R., Major, B., Epel, E. S. (2007). Why egalitarianism might be good for your health: Physiological thriving during stressful intergroup encounters. *Psychological Science, 18*, 991–998.

Mendes, W. B., & Koslov, K. (in preparation) *The ironic effects of attempting to control racial biases*.

Mendes, W. B., McCoy, S., Major, B., & Blascovich, J. (2008). How attributional ambiguity shapes physiological and emotional responses to social rejection and acceptance. *Journal of Personality and Social Psychology, 94*(2), 278–291.

Mendes, W. B., Major, B., McCoy, S., & Blascovich, J. (in press). How attributional ambiguity shapes physiological and emotional responses to social rejection and acceptance. *Journal of Personality and Social Psychology.*

Meyer, D. R. (1953). On the interaction of simultaneous responses. *Psychological Bulletin, 20*, 204–220.

Meyer, D. R., Bahrick, H. P., & Fitts, P. M. (1953). Incentive, anxiety, and the human blink rate. *Journal of Experimental Psychology, 45*, 183–287.

Milgram, S. (1963). Behavioral study of obedience. *Journal of Abnormal and Social Psychology, 67*, 371–378.

Miller, G. E., Dopp, J. M., Myers, H. F., Stevens, S. Y., & Fahey, J. L. (1999). Psychosocial predictors of natural killer cell mobilization during marital conflict. *Health Psychology, 18*, 262–271.

Moore, D. E., Kawagoe, S., Davajan, V., Nakamura, R. M., & Mischell, D. R. (1978). An in vivo system in man for quantitation of estrogenicity. II. Pharmacologic changes in binding capacity of serum corticosteroid-biding globulin induced by conjugated estrogens, mestranol, and ethinyl estradiol. *American Journal of Obstetrics and Gynecology, 130*, 482–486.

Motley, M. T., & Camden, C. T. (1988). Facial expression of emotion: A comparison of posed expressions versus spontaneous expressions in an interpersonal communications setting. *Western Journal of Speech Communication, 52*, 1–22.

Moyer, A. E., Rodin, J., Grilo, C. M., Cummings, N., Larson, L. M., & Rebuffe-Scrive, M. (1994). Stress-induced cortisol response and fat distribution in women. *Obesity Research, 2*, 255–262.

Moyna, N. M., Bodnar, J. D., Goldberg, H. R., Shurin, M. S., Robertson, R. J., & Rabin, B. S. (1999). Relation between aerobic fitness level and stress induced alterations in neuroendocrine and immune function. *International Journal of Sports Medicine, 20*, 136–141.

Murphy, M. C., Steele, C. M., & Gross, J. J. (2007). Signaling threat: How situational cues affect women in math, science, and engineering settings. *Psychological Science*, *18*(10), 879–885.

Obrist, P. A. (1981). *Cardiovascular psychophysiology: A perspective*. New York: Plenum Press.

Olsson, A., Ebert, J. P., Banaji, M. R., & Phelps, E. (2005). The role of social groups in the persistence of learned fear. *Science, 309*(5735), 785–787.

Otte, C., Hart, S., Neylan, T. C., Marmar, C. R., Yaffe, K., & Mohr, D. C. (2005). A meta-analysis of cortisol response to challenge in human aging: importance of gender. *Psychoneuroendocrinology*, 30, 80–91.

Oveis, C., Cohen, A., Gruber, J., Shiota, M., Haidt, J. & Keltner, D. (2009). Resting respiratory sinus arrhythmia is associated with tonic positive emotionality. *Emotion, 9,* 265–270.

Pennebaker, J. W., Hughes, C. F., & O'Heeron, R. C. (1987). The psychophysiology of confession: Linking inhibitory and psychosomatic processes. *Journal of Personality and Social Psychology, 52*(4), 781–793.

Peper, E., Wilson, V. S., Gibney, K. H., Huber, K., Harvey, R., & Shumay, D. M. (2003). The integration of electromyography (SEMG) at the workstation: Assessment, treatment, and prevention of repetitive strain injury (RSI). *Applied Psychophysiology and Biofeedback, 28*(2), 167–182.

Peters, M. L., Godaert, G. L., Ballieux, R. E., van Vliet, M., Willemsen, J. J., Sweep, F. C., & Heijnen, C. J. (1998). Cardiovascular and endocrine responses to experimental stress: Effects of mental effort and controllability. *Psychoneuroendocrinology, 23*, 1–17.

Phelps, E. A., O'Connor, K. J., Cunningham, W. A., Funayama, E. S., Gatenby, J. C., Gore, J. C., & Banaji, M. R. (2000). Performance on indirect measures of race evaluation predicts amygdala activation. *Journal of Cognitive Neuroscience, 12*, 729–738.

Porges, S. W. (2007). The polyvagal perspective. *Biological Psychology, 74*(2), 116–143.

Powers, S. I., Pietromonaco, P. R., Gunlicks, M., Sayer, A. (2006). Dating couples' attachment styles and patterns of cortisol reactivity in response to a relationship conflict. *Journal of Personality and Social Psychology, 90*(4), 613–628.

Priftis, K. N., Papadimitriou, A., Nicolaidou, P., & Chrousos, G. P. (2009). Dysregulation of the stress response in asthmatic children. *Allergy, 64*, 18–31.

Putnam, L. E., & Vanman, E. J. (1999). Long lead interval startle modification. In M. Dawson, A. M. Schell, & A. H. Böhmelt (Eds.), *Startle modification: Implications for neuroscience, cognitive science, and clinical science* (pp. 72–92). New York: Cambridge University Press.

Redwine, L. S., Altemus, M., Leong, Y. M., & Carter, C. S. (2001). Lymphocyte responses to stress in postpartum women: Relationship to vagal tone. *Psychoneuroendocrinology, 26*, 241–251.

Rimmele, U., Seiler, R., Marti, B., Wirtz, P. H., Ehlert, U., & Heinrichs, M. (2009). The level of physical activity affects adrenal and cardiovascular reactivity to psychosocial stress. *Psychoneuroendocrinology, 34*(2), 190–198.

Robbins, M. L., Dickerson, S. S., Epstein, E. B., & Zaldivar, F. (Under review). *The potential for future evaluation and cortisol: A preliminary investigation*.

Rohleder, N., Beulen, S. E., Chen, E., Wolf, J. M., & Kirschbaum, C. (2007). Stress on the dance floor: The cortisol stress response to social-evaluative threat in competitive ballroom dancers. *Personality and Social Psychology Bulletin, 33*, 69–84.

Rohleder, N., & Kirschbaum, C. (2006). The hypothalamic-pituitary-adrenal (HPA) axis in habitual smokers. *International Journal of Psychophysiology, 59*, 236–243.

Rohleder, N., Schommer, N. C., Hellhammer, D. H., Engel, R., & Kirschbaum, C. (2001). Sex differences in glucocorticoid sensitivity of proinflammatory cytokine production after psychosocial stress. *Psychosomatic Medicine, 63*, 966–972.

Rohleder, N., Wolf, J. M., Piel, M., & Kirschbaum, C. (2003). Impact of oral contraceptive use on glucocorticoid sensitivity of proinflammatory cytokine production after psychosocial stress. *Psychoneuroendocrinology, 28*, 261–273.

Rohrmann, S., Hennig, J., & Netter, P. (1999). Changing psychobiological stress reactions by manipulating cognitive processes. *International Journal of Psychophysiology, 33*, 149–161.

Roy, S. H., De Luca, C., Emley, M., Oddsson, L. I. E., Buijis, R. J. C., Levins, J., Newcombe, D. S., & Jabre, J. F. (1997). Classification of back muscle impairment based on the surface electromyographic signal. *Journal of Rehabilitation Research and Development, 34*, 405–414.

Sapolsky, R. M. (2005). The influence of social hierarchy on primate health. *Science, 308*, 648–652.

Scheepers, D. (2009). Turning social identity threat into challenge: Status stability and cardiovascular reactivity during intergroup competition. *Journal of Experimental Social Psychology, 45*, 228–233.

Schneider, K., & Unzner, L. (1992). Preschoolers' attention and emotion in an achievement and an effect game: A longitudinal study. *Cognition and Emotion, 6*, 37–63.

Schommer, N. C., Hellhammer, D. H., & Kirschbaum, C. (2003). Dissociation between reactivity of the hypothalamus-pituitary-adrenal axis and the sympathetic-adrenal-medullary system to repeated psychosocial stress. *Psychosomatic Medicine, 65*, 450–460.

Schwabe, L., Haddad, L., & Schachinger, H. (2008). HPA axis activation by a socially evaluated cold-pressor task. *Psychoneuroendocrinology, 33*(6), 890–895.

Schwartz, G. E., Fair, P. L., Salt, P., Mandel, M. R., & Klerman, G. L. (1976). Facial muscle patterning to affective imagery in depressed and nondepressed subjects. *Science, 192*, 489–491.

Seeman, T. E., Singer, B., Wilkinson, C. W., & McEwen, B. (2001). Gender differences in age-related change sin HPA axis reactivity. *Psychoneuroendocrinology, 26*, 225–240.

Seery, M. D., Blascovich, J., Weisbuch, M., & Vick, S. B. (2004). The relationship between self-esteem level, self-esteem stability, and cardiovascular reactions to performance feedback. *Journal of Personality and Social Psychology, 87*(1), 133–145.

Sephton, S. E., Sapolsky, R. M., Kraemer, H. C., Spiegel, D. (2000). Diurnal cortisol rhythm as a predictor of breast cancer survival. *Journal of the National Cancer Institute, 92*(12), 994–1000.

Shaw, W. A. (1940). The relation of muscular action potentials to imaginal weight lifting. *Archives of Psychology, 247*, 1–50.

Sherwood, A., Allen, M. T., Fahrenberg, J., Kelsey, R. M., Lovallo, W. R., & van Doornen, L. J. P. (1990). Methodological guidelines for impedance cardiography. *Psychophysiology, 27*, 1–23.

Sherwood, A., Dolan, C. A., & Light, K. C., (1990). Hemodynamics of blood pressure responses during active and passive coping. *Psychophysiology, 27,* 656–668.

Shimizu, A., & Inoue, T. (1986). Dreamed speech and speech muscle activity. *Psychophysiology, 23*, 210–215.

Shively, C.A., Laber-Laird, K., & Anton R.F. (1997). Behavior and physiology of social stress and depression in female cynomolgus monkeys. *Biological Psychiatry, 41*(8), 871–82.

Simons, R. F., & Zelson, M. F. (1985). Engaging visual stimuli and reflex modification. *Psychophysiology, 22*, 44–49.

Sloan, R. P., Bagiella, E., & Shapiro, P. A. (2001). Hostility, gender, and cardiac autonomic control. *Psychosomatic Medicine, 63*(3), 434–440.

Sothmann, M. S., Gustafson, A. B., Garthwaite, T. L., Horn, T. S., & Hart, B. A. (1988). Cardiovascular fitness and selected adrenal hormone responses to cognitive stress. *Endocrine Research, 14*, 59–69.

Spath-Schwalbe, E., Uthgenannt, D., Voget, G., Kern, W., Born, J., & Fehm, H. L. (1993). Corticotripin-releasing hormone-induced adrenocorticotropin and cortisol secretion depends on sleep and wakefulness. *Journal of Clinical Endocrinology & Metabolism, 77*, 1170–1173.

Spiegel, K., Leproult, R., & Van Cauter, E. (1999). Impact of sleep debt on metabolic and endocrine function. *Lancet, 354*, 1435–1439.

Starcke, K., Wolf, O. T., Markowitsch, H. J., & Brand, M. (2008). Anticipatory stress influences decision-making under explicit risk conditions. *Behavioral Neuroscience, 122*(6), 1352–1360.

Steptoe, A., Cropley, M., Griffith, J., & Kirschbaum, C. (2000). Job strain and anger expression predict early morning elevations in salivary cortisol. *Psychosomatic Medicine, 62*, 286–292.

Steptoe, A., Fieldman, G., Evans, O., & Perry, L. (1993). Control over work pace, job strain and cardiovascular responses in middle-aged men. *Journal of Hypertension, 11*, 751–759.

Steptoe, A., Fieldman, G., Evans, O. & Perry, L. (1996). Cardiovascular risk and responsivity to mental stress: The influence of age, gender, and risk factors. *Journal of Cardiovascular Risk, 3*, 83–93.

Stern, R. M., Ray, W. J., & Quigley, K. S. (2003). Psychophysiological recording. *Psychophysiology, 40*(2), 314–315.

Stone, A. A., Schwartz, J. E., Smyth, J., Kirschbaum, C., Cohen, S., Hellhammer, D., & Grossman, S. (2001). Individual differences in the diurnal cycle of salivary free cortisol: A replication of flattened cycles for some individuals. *Psychoneuroendocrinology, 26*(3), 295–306.

Stritzke, W. G. K., Patrick, C. J., & Lang, A. R. (1995). Alcohol and human emotion: A multidimensional analysis incorporating startle-probe methodology. *Journal of Abnormal Psychology, 104*, 114–122.

Stroud, L. R., Salovey, P., & Epel, E. S. (2002). Sex differences in stress responses: Social rejection versus achievement stress. *Biological Psychiatry, 52*, 318–327.

Stroud, L. R., Tanofsky-Kraff, M., Wilfley, D. E., & Salovey, P. (2000). The Yale Interpersonal Stressor (YIPS): Affective, physiological, and behavioral responses to a novel interpersonal rejection paradigm. *Annals of Behavioral Medicine, 22*, 204–213.

Swenson, R. M. & Vogel, W. H. (1983). Plasma catecholamines and corticosterone as well as brain catecholamines changes during coping in rats exposed to footshock. *Pharmacology, Biochemistry, & Behavior, 18*, 689–693.Tassinary, L. G., & Cacioppo, J. T. (1992). Unobservable facial actions and emotion. *Psychological Science, 3*(1), 28–33.

Tassinary, L. G., Cacioppo, J. T., & Geen, T. R. (1989). A psychometric study of surface electrode placements for facial electromyographic recording: I. The brow and cheek muscle regions. *Psychophysiology, 26*, 1–16.

Tassinary, L. G., Cacioppo, J. T., & Vanman, E. J. (2007). The skeletomotor system: Surface electromyography. In J. T. Cacioppo, L. G. Tassinary, & G. G. Berntson (Eds.), *Handbook of psychophysiology* (3rd ed., pp. 267–299). Cambridge, UK: Cambridge University Press.

Tassinary, L. G., Orr, S. P., Wolford, G., Napps, S. E., & Lanzetta, J. T. (1984). The role of awareness in affective information processing: An exploration of the Zajonc hypothesis. *Bulleting of the Psychonomic Society, 22*(6), 489–492.

Tattersall, A. J., & Hockey, G. R. (1995). Level of operator control and changes in heart rate variability during simulated flight maintenance, *Human Factors, 37*, 682–698.

Taylor, S. E., Welch, W. T., Kim, H., & Sherman, D. K. (2007). Cultural differences in the impact of social support on psychological and biological stress responses. *Psychological Science, 18*(9), 831–837.

Tomaka, J., & Blascovich, J. (1994). Effects of justice beliefs on cognitive appraisal of and subjective physiological, and behavioral responses to potential stress. *Journal of Personality and Social Psychology*, *67*(4), 732–740.

Tomaka, J., Blascovich, J., Kelsey, R. M., & Leitten, C. L. (1993). Subjective, physiological, and behavioral effects of threat and challenge appraisal. *Journal of Personality and Social Psychology, 65*, 248–260.

Trepman, E., Gellman, R. E., Solomon, R., Murthy, K. R., Micheli, L. J., & De Luca, C. (1994). Electromyographic analysis of standing posture and demi-plié in ballet and modern dancers. *Medicine and Science in Sports and Exercise, 26*, 771–782.

Tsuda, A., Steptoe, A., West, R., Fieldman, G., & Kirschbaum, C. (1996). Cigarette smoking and psychophysiological stress responsiveness: Effects of recent smoking and temporary abstinence. *Psychopharmacology, 126*, 226–233.

Valins, S. (1967). Cognitive effects of false heart-rate feed-back. *Journal of Personality and Social Psychology, 4,* 400–408.

Vallacher, R.R., Read, S.J., & Nowak, A. (2002). The dynamical perspective in personality and social psychology. *Personality and Social Psychology Review, 6*(4), 264–273.

Van Cauter, E., Leproult, R., & Kupfer, D. J. (1996). Effects of gender and age on the levels and circadian rhythmicity of plasma cortisol. *Journal of Clinical Endocrinology & Metabolism, 81*(7), 2468–2473.

van der Pompe, G., Antoni, M. H., & Heijnen, C. J. (1996). Elevated basal cortisol levels and attenuated ACTH and cortisol responses to a behavioral challenge in women with metastatic breast cancer. *Psychoneuroendocrinology, 21*, 361–374.

van Eck, M. M., Nicolson, N. A., Berkhof, H., & Sulon, J. (1996). Individual differences in cortisol responses to a laboratory speech task and their relationship to responses to stressful daily events. *Biological Psychology, 43*, 69–84.

Vanman, E. J., Boehmelt, A. H., Dawson, M. E., & Schell, A. M. (1996). The varying time courses of attentional and affective modulation of the startle eyeblink reflex. *Psychophysiology, 33*(6), 691–697.

Vanman, E. J., Paul, B. Y., Ito, T. A., & Miller, N. (1997). The modern face of prejudice and structural features that moderate the effect of cooperation on affect. *Journal of Personality and Social Psychology, 73*(5), 941–959.

Vanman, E. J., Saltz, J. L., Nathan, L. R., & Warren, J. A. (2004). Racial discrimination by low-prejudiced Whites facial movements as implicit measures of attitudes related to behavior. *Psychological Science, 15*(11), 711–714.

Veldhuis, L. D., Iranmanesh, A., Johnson, M L., & Lizarralde, G. (1990). Amplitude, but not frequency, modulation of adrenocorticotropin secretory bursts gives rise to the nyctohemeral rhythm of the corticotropic axis in man. *Journal of Clinical Endocrinology & Metabolism, 71*, 452–463.

Venables, P. H., & Christie, M. J. (1973). Mechanisms, instrumentation, recording echniques, and quantification of responses. In W. F. Prokasy, & D. C. Raskin (Eds.), *Electrodermal activity in psychological research* (pp. 1–124). New York: Academic Press.

Vgontzas, A. N., Bixler, E. O., Lin, H. M., Prolo, P., Mastorakos, G., Vela-Bueno, A., Kales, A., & Chrousos, G. P. (2001). Chronic insomnia is associated with nyctohemeral

activation of the hypothalamic-pituitary-adrenal axis: Clinical implications. *Journal of Clinical Endocrinology & Metabolism, 86*, 3787–3794.

Vgontzas, A. N., Mastorakos, G., Bixler, E. O., Kales, A., Gold, P. W., Chrousos, G. P. (1999). Sleep deprivation effects on the activity of the hypothalamus-pituitary-adrenal and growth axes: Potential clinical implications. *Clinical Endocrinology, 51*, 205–215.

Vrana, S. R. (1993). The psychophysiology of disgust: Differentiating negative emotional contexts with facial EMG. *Psychophysiology, 30*(3), 279–286.

Vrana, S. R., Spence, E. L., & Lang, P. J. (1988). The startle probe response: A new measure of emotion? *Journal of Abnormal Psychology, 97*, 487–491.

Waersted, M., & Westgaard, R. H. (1966). Attention-related muscle activity in different body regions during VDU work with minimal physical activity. *Ergonomics, 39*, 661–676.

Wegner, D. M., Broome, A., & Blumberg, S. J. (1997). Ironic effects of trying to relax under stress. *Behaviour Research and Therapy, 35*(1), 11–21.

Weibel, L., Follenius, M., Spiegel, K., Ehrhart, J., & Brandenberger, G. (1995). Comparative effect of night and daytime sleep on the 24-hour cortisol secretory profile. *Sleep, 18*, 549–556.

Weiner, H. (1992). *Perturbing the organism: The biology of stressful experience*. Chicago, IL: University of Chicago Press.

Weiss, J. M. (1971). Effects of coping behavior in different warning signal conditions on stress pathology in rats. *Journal of Comparative and Physiological Psychology, 77*, 1–13.

Willard, G. & Gramzow, R. (2008). Exaggeration in memory: Systematic distortion of self-evaluative information under reduced accessibility. *Journal of Experimental Social Psychology*, 44, 246–259.

Winkielman, P., & Cacioppo, J. T. (2001). Mind at ease puts a smile on the face: Psychophysiological evidence that processing facilitation elicits positive affect. *Journal of Personality and Social Psychology, 81*(6), 989–1000.

Wolf, K., Mass, R., Ingenbleek, T., Kiefer, F., Naber, D., & Wiedemann, K. (2005). The facial pattern of disgust, appetence, excited joy, and relaxed joy: An improved facial EMG study. *Scandinavian Journal of Psychology, 46*, 403–409.

Wolf, O. T., Schommer, N. C., Hellhammer, D. H., McEwen, B. S., & Kirschbaum, C. (2001). The relationship between stress induced cortisol levels and memory differs between men and women. *Psychoneuronedocrinology, 26*, 711–720.

Woodworth, R. S., & Schlosberg, H. (1954). *Experimental psychology* (2nd ed.). New York: Holt.

Wright, R. A., & Kirby, L. D. (2003). Cardiovascular correlates of challenge and threat appraisals: A critical examination of the biopsychosocial analysis. *Personality and Social Psychology Review*, 7, 216–233.

Wust, S., Federenko, I., Hellhammer, D. H., & Kirschbaum, C. (2000). Genetic factors, perceived chronic stress, and the free cortisol response to awakening. *Psychoneuronendocrinology, 25*, 707–720.

Yehuda, R. (2006). Advances in understanding neuroendocrine alterations in PTSD and their therapeutic implications. *Annals of the New York Academy of Sciences, 1071*, 137–166.

Yim, I. S., Quas, J. A., Cahill, L., & Hayakawa, C. M. (2010). Children's and adults' salivary cortisol responses to an identical psychosocial laboratory stressor. *Psychoneuroendocrinology, 5*(2), 241.