Article

Incorporating neuroendocrine methods into intergroup relations research

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Abstract

Intergroup researchers have the opportunity to access to a wide variety of methods to help deepen theoretical insights about intergroup relations. In this paper, we focus on neuroendocrine measures, as these physiological measures offer some advantages over traditional measures used in intergroup research, are noninvasive, and are relatively easy to incorporate into existing intergroup paradigms. We begin by discussing the major neuroendocrine systems in the body and their measurable biological products, emphasizing systems that have conceptual relevance to intergroup relations. We then describe how to collect, store, and quantify neuroendocrine measures. Altogether, this paper serves as a primer for intergroup researchers interested in adding neuroendocrine measures to their methodological toolkits to enrich the study of intergroup relations.

Neuroendocrine Methods in Research on Intergroup Relations

It is clear that the human body is responsive to myriad environments, including but not limited to intergroup contexts. In this paper, we discuss how furthering our understanding of human physiology, specifically neuroendocrinology, can be beneficial to the study of intergroup relations. Our goal is to show how incorporating neuroendocrine measures into intergroup research can offer new ways of understanding the interaction of psychology and physiology in intergroup contexts.

Neuroendocrinology is the study of how the nervous system interacts with the endocrine

system to sustain life. Both the nervous and endocrine systems are the mediums through which cells communicate with each other, with the nervous system communicating quickly

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through nerves and the endocrine system communicating relatively slowly by releasing hormones and other biological products into bodily fluids (e.g., saliva, blood, urine). As the endocrine system developed across the phylogenetic chain, so too did intergroup behavior (e.g., Southwick, 1962). Nonetheless, we know relatively little about how neuroendocrine systems react to intergroup stimuli or how they may augment or inhibit normal intergroup processes.

There are numerous benefits to understanding the links between neuroendocrinology and intergroup processes. For one, like most physiological responses, neuroendocrine responses are not susceptible to self-report biases. While one can edit what is said or reported on a questionnaire, it is very difficult—if not impossible—to control, for instance, the activity of one's salivary glands. Given that intergroup research typically examines sensitive topics, such as bias and stigma, the value of examining bodily responses, in addition to more subjective self-reports, is heightened in these contexts where individuals may be unwilling or unable to report their true feelings (Mendes, 2009).

Another benefit of neuroendocrine measures is that, within the realm of psychophysiological methods, neuroendocrine measures are more readily incorporated into intergroup experiments than peripheral electrophysiological measures (e.g., heart rate, skin conductance) or intracranial measures (e.g., fMRI, electroencephalogram). Neuroendocrine measures do not require participants to be tethered to physiological hardware by electrical wires, whereas most electrophysiological hardware does. Because participants are not directly connected to physiological equipment, the use of neuroendocrine measures allows participants to behave in a more natural manner while they are completing experimental tasks. This feature also facilitates study designs that require participants to be actively mobile. Salivary neuroendocrine measures are also less invasive than electrophysiological measures, because the latter measures require direct physical contact with participants when placing sensors on their skin; hormones can be collected without touching the participant. Especially relevant to researchers interested in intergroup contact,

intergroup interaction, and minimal groups, it is much easier to measure hormones from many participants at once than to collect and synchronize electrophysiological measures from multiple participants. For these reasons, neuroendocrinology is a particularly attractive branch of psychophysiology for intergroup research.

One final benefit stems from the fact that the field of intergroup relations has evolved dramatically over the past decade, making it ripe for incorporating interdisciplinary insights from fields including psychology, economics, and neuroscience to help extend intergroup theories and add greater precision to intergroup research. Since developmental differences in the major neuroendocrine systems may covary in meaningful ways with the complexity of social behavior across species, incorporating neuroendocrinology into intergroup research can allow intergroup researchers to draw upon extant research on intergroup behavior in nonhuman primates and other animals (e.g., Southwick, 1962).

The objective of this paper is to introduce intergroup researchers to neuroendocrinological concepts and methods as they apply to the study of intergroup relations. This paper is intended as a primer for researchers who have research questions pertaining to the hormonal correlates of intergroup processes. We begin with an overview of the constructs that are the meeting point of intergroup relations and neuroendocrinology and discuss the specific neuroendocrine measures that are relevant to each domain. We then lead the reader through the process of collecting hormones and turning them into valuable data that can be analyzed statistically. We close with a discussion of the psychological interpretation of neuroendocrine findings.

Neuroendocrine Systems and Their Relevance to Intergroup Relations

There are several possible domains in which the use of neuroendocrine measures may provide novel insights into key phenomena related to intergroup relations. We focus on the domains of stress, health, dominance, and affiliation. We have

selected these domains as they apply to some of the key puzzles remaining in intergroup relations research. Intergroup researchers have long been interested in understanding the stress associated with intergroup interactions (e.g., Stephan & Stephan, 1985). Further, certain minority groups are more exposed to stress than majority groups due to discrimination and stigmatization, propelling research on ethnic health disparities (Mays, Cochran, & Barnes, 2007). Additionally, in intergroup relations, competition (Sherif, Harvey, White, Hood, & Sherif, 1961) and dominance (Sidanius & Pratto, 2001) are among the most widely researched topics. More recently, intergroup researchers have been interested in the positive side of intergroup relations, with a focus on the factors that help or hinder affiliation among ingroup and outgroup members (de Dreu, 2012; Page-Gould, Mendoza-Denton, & Tropp, 2008). Importantly, each of these core constructs in intergroup research are topics that are widely researched within neuroendocrinology, opening up avenues for interdisciplinary insights to be shared that can enhance intergroup theories. In the section that follows, we will review five neuroendocrine measures that have been used to examine these constructs, specifically cortisol, dehydroepiandrosterone, testosterone, oxytocin, and alpha-amylase.

Stress

Stress has been defined as an adaptive response to a demanding situation (Selye, 1950). Physiologically, stress occurs when the body is forced to change in order to maintain homeostasis. An adaptive stress response is one where, in response to a stressor, the body activates the stress systems quickly and then readily returns to resting levels after the stressor (McEwen, 1998). A maladaptive stress response is one where the body takes longer to return to homeostasis following a stressor.

The two main physiological systems associated with stress responses are the sympathetic-adrenal-medullary (SAM) and the hypothalamic-pituitary-adrenocortical (HPA). A person's pattern of SAM and HPA activation in response to stress predicts whether they will perform well or poorly while stressed. When the SAM axis is activated but the HPA is not activated, stress appears to enhance performance (Blascovich, Mendes, Tomaka, Salomon, & Seery, 2003; Dienstbier, 1989). However, when the SAM and HPA axes are both activated, stress may inhibit peak performance (Blascovich et al., 2003). These principles have been applied to intergroup contexts in an effort to differentiate between stress responses of intergroup interaction partners. Individuals interacting with nonstigmatized partners were likely to exhibit cardiovascular responses reflecting SAM activation alone, but those who interacted with a stigmatized partner exhibited physiological patterns consistent with coactivation of the SAM and HPA (Blascovich, Mendes, Hunter, Lickel, & Kowai-Bell, 2001). Intergroup researchers can use neuroendocrine measures that index SAM and HPA axis activation as one way to examine stress states during intergroup interactions. HPA activation is most typically measured with the hormone cortisol, and SAM activation can be measured with the peptide alpha-amylase. The HPA axis also releases the hormone dehydroepiandrosterone (DHEA) along with cortisol, and researchers interested in resilient responses to stress are becoming increasingly interested in the ratio of DHEA to cortisol during HPA activation.

Cortisol. Commonly known as the "stress hormone," cortisol is released in response to physical and psychological stressors (Selye, 1936, 1950). Cortisol indexes activation of the HPA and is present in saliva approximately 20 minutes after the HPA activates. Cortisol is particularly reactive to situations that are uncontrollable or characterized by social-evaluative threat (Dickerson & Kemeny, 2004), thus it can offer insight into the degree to which an intergroup situation is stressful. Moreover, cortisol measures reflect psychological processes that are relevant to both minority- and majority-group members. For instance, cortisol has been found to increase during intergroup interactions with outgroup strangers, particularly among individuals who are either implicitly prejudiced or concerned about race-based rejection (Page-Gould et al., 2008).

Interestingly, this effect was attenuated over repeated intergroup interactions with the same outgroup member, thus reductions in cortisol over time may reflect habituation in intergroup contexts.

DHEA. DHEA and its sulphate form, dehydroepiandrosterone-sulphate (DHEA-S), are also released by the HPA axis in response to stress, but operate very differently from cortisol. Levels of DHEA-S in the saliva reflect activation of the HPA axis approximately 20 minutes before collection. While cortisol breaks down molecules to create a surge of glucose to fuel the body, DHEA responds to stress by binding molecules together to create more complex proteins. Perhaps because of this process, DHEA appears to protect the body against some of the wear and tear caused by cortisol release. Over the last two decades, social neuroendocrinologists have been investigating the role of DHEA in resilience and recovery from stress (Epel, McEwen, & Ickovics, 1998). During stressful intergroup interactions, low implicit prejudice predicts relatively greater increases in DHEA-S than cortisol (Mendes, Gray, Mendoza-Denton, Major, & Epel, 2007). Therefore, researchers interested in positive responses to stress should consider measuring both cortisol and DHEA to understand the role they jointly play in potentially stressful intergroup contexts.

Salivary alpha-amylase (sAA). SAM activation can be measured through changes in the protein alpha-amylase in the saliva. As the SAM axis acts faster than the HPA axis, levels of sAA reflect SAM activity 5 minutes prior to collection. sAA has been shown to increase during intergroup interactions regardless of individual differences such as motivation to control prejudice (Trawalter, Adam, Chase-Lansdale, & Richeson, 2012). One question that emerges from intergroup research that has used cortisol and sAA is whether intergroup interactions elicit adaptive stress responses (e.g., SAM activation) in most individuals, but more maladaptive responses (e.g., SAM and HPA activation) in individuals with implicit

biases—a question that can be addressed through continued use of these measures in intergroup research

Dominance and Aggression

Issues of group status and dominance are pervasive in intergroup processes. For example, minority- and majority-group members can perceive intergroup interactions quite differently (Tropp, Stout, Boatswain, Wright, & Pettigrew, 2006). In addition, beliefs about the inevitability of social hierarchies predict a wide range of intergroup attitudes and behavior (Sidanius & Pratto, 2001). At the extreme end of intergroup conflict is intergroup aggression and violence. As power and status play a fundamental role in intergroup processes (Goodwin, Operario, & Fiske, 1998), neuroendocrine measures related to dominance and aggression may be of special interest to intergroup researchers.

For the most part, dominance and aggression have been most comprehensively studied in relation to the hypothalamic-pituitary-gonadal (HPG) reproductive axis, which releases the steroid hormone testosterone. For instance, injections of testosterone in birds led to increased aggression and dominance among high-status birds but caused no increase in aggressive and dominant behavior among low-status animals when high-status birds were present (Collias, Barfield, & Tarvyd, 2002; Josephs, Sellers, Newman, & Mehta, 2006). This suggests that the HPG axis relates differently to aggression based on the social context (cf. Sapolsky, 1991). Recent advances in social neuroendocrinology have offered evidence that the HPG and HPA axes should be measured concurrently in humans to understand the relationship between the HPG and dominance (Mehta & Josephs, 2010; Sapolsky, 1991).

Testosterone. Testosterone is an anabolic steroid hormone produced by both the testes and the adrenal cortex. Like cortisol and DHEA, testosterone levels in the saliva reflect circulating levels approximately 20 minutes before saliva collection (Dabbs, 1993). Testosterone is associated with the

desire to gain or maintain status and responds to competitive situations (Mazur & Booth, 1998; Mehta & Josephs, 2006). Testosterone levels also appear to rise after winning a competition and fall after losing a competition (Mehta & Josephs, 2006). Interestingly, it is not precompetition testosterone that is related to the outcome of the competition, but rather postcompetition testosterone seems to reflect the dominance hierarchy that emerges from the competition (Mehta & Josephs, 2006; Oliveira, Gouveia, & Oliveira, 2009).

Recently, social neuroendocrinologists have begun to explore the relationship between reactivity in the HPG and HPA axes. For example, the dual-hormone hypothesis (Mehta & Josephs, 2010) posits that testosterone's relationship with dominance depends on activation of the HPA axis. In numerous studies, these researchers found that testosterone predicts dominance when cortisol reactivity is low, indicating that the HPA axis is relatively inactive. In contrast, when cortisol reactivity is high, testosterone is either unrelated or negatively related to dominance. The dual-hormone hypothesis is based on the premise that elevated HPA activity signals threat or unstable environments in which dominance behaviors are inhibited because they may not be effective at increasing social status during these high-stress times. However, in a safe or stable environment, testosterone may facilitate status-seeking, dominant behaviors. Thus, intergroup researchers who are interested in understanding how power, status, and competition relate to intergroup dynamics should consider measuring both testosterone and cortisol in their investigations.

Health

Understanding the role that discrimination plays in ethnic health disparities has been a focus of intergroup relations research for decades (e.g., Clark, Anderson, Clark, & Williams, 1999). A large and growing body of research has demonstrated that African Americans tend to be more exposed to stressors than other ethnic groups (Neblett, Shelton, & Sellers, 2004; Sellers, Caldwell, Schmeelk-Cone, & Zimmerman, 2003;

Sellers & Shelton, 2003). This exposure to stress has been attributed to the discrimination that comes from being a member of a highly visible, isolated, and marginalized minority group. Epidemiological studies indicate that people who are routinely exposed to discrimination are at a greater risk for mental health problems, such as depression, as well as physical health problems, such as hypertension, coronary heart disease, and stroke (Jackson et al., 2004; Singer & Ryff, 1999). Compared to European Americans, African Americans have shorter life expectancies, higher infant mortality, and are two times more likely to be hypertensive (Fiscella & Williams, 2004; Flack et al., 1995; Williams, Yu, Jackson, & Anderson, 1997).

Neuroendocrine measures that index chronic stress are increasingly being used to document these health disparities and enact preventative interventions (Mays et al., 2007). While acute stress can have positive or negative repercussions, the effects of chronic stress are almost always negative. Chronic stress is manifested physiologically when the body's stress systems become consistently activated or dysregulated (Juster, McEwen, & Lupien, 2010; McEwen, 2004). HPA dysregulation is most commonly measured through changes in cortisol in the first hour after waking.

Cortisol awakening response (CAR). The CAR refers to the trajectory of cortisol levels in the hour after waking (Pruessner et al., 1997). Consistent with natural changes in cortisol over the course of the day, an individual with a healthy, well-regulated HPA axis will exhibit a sharp rise in cortisol levels between the time of waking and 30 minutes after waking. This cortisol peak should then drop sharply, ideally returning to waking levels or even dropping below waking levels within 60 minutes after waking. When the HPA axis is dysregulated, cortisol levels tend not to rise as high in the first 30 minutes after waking and not to fall as low as waking levels within 60 minutes postwakening. In other words, a well-regulated HPA axis will be marked by a steep slope up between Minutes 0 through 30 and a steep slope down between

Minutes 30 and 60. A dysregulated HPA axis will be marked by a blunted cortisol increase in the first 30 minutes that is nonetheless sustained past the first hour of waking.

Although to date there is little research that utilizes CAR to better understand the dynamics of intergroup interactions, much insight can be gained from more broadly examining how life events can influence CAR. For instance, in a longitudinal study of older adults, Adam, Hawkley, Kudielka, and Cacioppo (2006) found that prior-day feelings of loneliness, sadness, threat, and lack of control were associated with a greater volume of cortisol released in the hour after waking the following day. This research suggests that negative emotions and psychosocial states that can be elicited by discrimination may help account for the chronic stress certain ethnicities experience relative to others. Further, this research suggests that coping strategies that heighten feelings of affiliation and minimize emotions associated with rejection may buffer health for stigmatized individuals and targets of discrimination.

Affiliation

Why do we bond with similar others and exclude dissimilar others? The tendency to affiliate with similar others, also known as homophily, is observed across both human (McPherson, Smith-Lovin, & Cook, 2001) and animal species (Fu, Nowak, Christakis, & Fowler, 2012). Notably, even scientists publish with coauthors from the same ethnic group at a rate higher than expected by chance, even though papers with ethnically diverse coauthors have higher citation counts (Freeman & Huang, 2014). To understand the processes that perpetuate homophily, it is important to determine the biological processes that are common to all species that exhibit this behavior. The hormone peptide oxytocin plays a key role in social bonding among animals, and thus many researchers have turned to oxytocin to examine affiliation and exclusion tendencies.

Oxytocin. Colloquially called "the attachment hormone," oxytocin has been implicated in behaviors associated with the tendency to "tend and befriend" under conditions of stress (Taylor, 2006). Among monogamous mammals (e.g., prairie voles), oxytocin receptors co-occur with dopamine receptors. However, this brain structure is not observed among nonmonogamous mammals (e.g., mountain voles), which helps explain these differential mating patterns (Insel, Young, & Wang, 1997). In humans, oxytocin plays a critical role in biological processes associated with mating and nurturing offspring. Oxytocin mediates the release of breast milk among nursing mothers, and the largest oxytocin spike experienced by most women occurs at the time of childbirth. Additionally, both men and women show large oxytocin reactivity during sexual orgasm. As a result of these properties, neuroendocrinologists have become increasingly interested in the role oxytocin plays in social processes.

In a seminal study, Kosfeld, Heinrichs, Zak, Fischbacher, and Fehr (2005) demonstrated that intranasal administration of oxytocin resulted in greater trust in strangers as measured through behaviors in a collective resource-allocation game. De Dreu et al. (2010) extended this work to the intergroup domain by systematically varying whether the strangers were ethnic ingroup or outgroup members. Counter to the idea that oxytocin globally increases trust in humans, oxytocin administration resulted in greater trust in ingroup members but systematically *less* trust in outgroup members (de Dreu et al., 2010). These findings suggest that oxytocin may accentuate homophilous tendencies. Additional studies of this nature will help intergroup researchers to build more precise theories around the biological, psychological, and social mechanisms that can influence the degree to which individuals create bonds and engage in trusting behaviors with similar and dissimilar others.

Summary

Up to this point, we have given a brief overview of psychophysiological theories, neuroendocrine systems, and neuroendocrine measures. We have also speculated on possible domains of intergroup

research in which the use of neuroendocrine measures may provide novel insights for intergroup scholars. We now turn to the methodologies for collecting these neuroendocrine measures and discuss some of the challenges associated with using these measures in intergroup research.

Measuring Neuroendocrine Responses

Relative to other psychophysiological measures, adding neuroendocrinology to one's research toolkit requires relatively little new equipment. This section will give an overview of the process of collecting hormones and converting them to data that can be statistically analyzed. We begin by describing hormone collection in detail. Next, we discuss the experimental design implications for measuring hormones, participant screening criteria, and prelab behavioral restrictions. We then conclude this section by discussing how to properly address the safety concerns that are unique to the collection and storage of saliva.

Collecting Neuroendocrine Data

The most common method for measuring hormones is through the collection of saliva, blood, or urine. The majority of psychology labs incorporating neuroendocrine measures collect saliva as an accessible method that is conducive to experimental research designs. There is inherent physical risk to collecting blood since it can only be collected invasively (i.e., skin puncture). Thus, most institutions require blood draws to be conducted by a registered nurse or in a hospital setting, making blood collection resource-intensive. Although urine collection involves less physical risk than collecting blood, these samples can be difficult to incorporate into common social psychological paradigms as they require interrupting the experiment to escort participants to the bathroom. We therefore focus on salivary methods in this paper. The typical saliva collection procedure entails collecting saliva at specified periods during a study, storing this saliva in a subzero freezer until the study has been completed, and then sending the saliva on dry ice in a batch to be assayed by a saliva testing company for the specific hormone(s) of interest.

Saliva collection techniques. There are three key saliva collection methods. *Passive drool* requires participant to "expectorate" or drool into a 2-mL test tube through a straw. These test tubes must be able to be frozen, so they are called "cryovials." Another common expectoration method is to have participants *spit* into a 5- to 50-mL cryovial after allowing saliva to collect at the base of their mouths for 5 minutes without swallowing. This larger cryovial prevents participants from missing the tube when they expectorate. The use of an *oral swab* is a third method of saliva sampling where an absorbent piece of cotton is placed in the mouth for a set period of time.

For both expectoration methods, it is typical to collect 1 mL of saliva, as most assay processes require 100 μL to assay each hormone of interest and another 100 μL to evaluate reliability of the assay. Given some participants have trouble drooling, it is good practice to try to collect .5 mL for each hormone of interest, so that you will still have enough saliva to assay if the participant provides less than the target amount. Since saliva is highly viscous, it is common for bubbles to form at the top of the cryovial, minimizing the desired saliva quantity. We recommend asking participants to lightly tap the bottom of the test tube on a hard surface periodically while they are expectorating. This minor tension will break the bubbles and facilitate the collection of a full sample.

The oral swab technique accounts for issues regarding flow rate of saliva and helps filter mucus and other matter from the sample. However, many avoid this approach as hormone levels can be artificially inflated when collected with swabs. For example, testosterone levels are almost twice as high when measured with oral swabs compared to expectoration (Dabbs, 1993). Due to the potential of cotton corrupting the sample, many researchers use the expectoration methods. Further, participants sometimes complain that they do not like the taste or feeling of the cotton, so the expectoration method also

appears to be less invasive in an experiential sense.

Sample labeling and safe handling. Most companies specializing in saliva testing (e.g., Salimetrics, IBL-Hamburg) provide cryovials and oral swab tubes for saliva collection that are made of high-quality polypropylene to avoid problems with analyte retention or the introduction of contaminants. Some even provide labels that can be ordered in advance by the researchers with specific barcodes and ID numbers for each study participant. If labels are not ordered in advance, it is sufficient to create labels using a thin-tipped permanent marker, as the ink from nonpermanent markers may bleed during the thawing process that occurs during assay. Given that bodily fluids are being collected, experimenters that handle saliva samples must use nonporous gloves (e.g., nitrile, latex) while collecting each sample. Additionally, gloves should be used to transfer the saliva to the freezer at the end of the session. Since some experiments include two or more saliva samples, it is fine to keep samples in a cryovial rack at room temperature throughout the experiment then transfer them to the freezer all at once at the end of the experimental session.

Trouble expectorators. Many participants find it easy to provide 1 mL of saliva through the expectoration method. A person who drools quickly can provide a 1 mL sample in about 1 minute, but a person who has dry mouth can take much longer (e.g., 10 or 15 minutes). There are at least three activities that can facilitate efficient expectoration. The physical symptom of slowed flow rate is the feeling of a dry mouth. To combat this potential cause, participants may be provided with a 4-oz. glass of water at the beginning of the experiment. Individuals who continue to have difficulty while expectorating can be guided through a meditation on salivation. Direct their attention to the salivary ducts on the side of the tongue and gums. Ask them to imagine the feeling of water flowing through each of these ducts like a faucet and then pooling in the base of the mouth, in the space under the tongue. The key to the salivation meditation is to focus awareness on the feeling of expectorating to stimulate expectoration. It can also be helpful to ask participants to stroke right above their jaw line with their pointer finger to stimulate their salivary glands. Finally, some researchers ask participants to chew sugar-free gum to stimulate expectoration.

Saliva collection is simple and flexible. Expectoration methods can be incorporated into a variety of intergroup paradigms. Saliva samples can be collected in the laboratory or the field, and they add only about 5 minutes per sample to the overall experiment duration. Moreover, saliva can be collected from as many people simultaneously as there are the cryovials to support collection, making neuroendocrine methods highly attractive for social interactions between two or more people. Once saliva samples have been collected, they need to be stored frozen until they can be shipped to a lab to quantify hormone levels from the saliva samples.

Storing saliva samples. All hormones described in this paper need to be frozen if the hormone values will not be extracted from the sample immediately. A standard −20 °C freezer is sufficient for storing saliva samples for up to 9 months (Gröschl, Wagner, Rauh, & Dörr, 2001; Kirschbaum & Hellhammer, 1989), but levels of hormones in the samples are likely to degrade after that point. However, −80 °C freezers provide archival storage, such that samples can be stored indefinitely at this temperature and shipped for assay all at once.

It is important to avoid thawing saliva samples after first freezing them, as multiple freeze–thaw cycles can affect the hormones described here, but at different rates. Regardless of whether you purchase a −20 °C or −80 °C freezer, you will want to talk to your institution about powering your freezer through their backup electrical generator. Most likely, this will involve some degree of construction to feed the backup electricity line into your laboratory. Prior to this construction, make sure that you ask for the correct type of outlet to be installed, because many freezers use a special type of plug called a "winking eye."

Like any piece of equipment, freezers can fail. Freezer failure can have catastrophic impact on your study should your samples be destroyed. Every lab should have a protocol in place to respond to a freezer failure. This protocol will include backup methods to maintain sample temperature in the case of an emergency (e.g., quickly transferring them to a cooler with dry ice) and contact information for technicians associated with the freezer company. Freezer failures can be prevented through annual freezer maintenance.

Shipping saliva samples. Typically, dry ice is used to ship frozen saliva samples to the saliva-testing lab. Dry ice evaporates directly from a solid to a gas at −78.5 ℃, thus it keeps samples as cold as they would be in a −80 ℃ freezer. Dry ice can be purchased at local gas supply companies, many of which deliver although some require the dry ice to be picked up directly from their facility. Researchers should consider purchasing a plastic cooler to transport dry ice to and from the lab, as disposable Styrofoam coolers will crack from the temperature.

Dry ice should be handled with work gloves, as it sticks to and burns the skin almost immediately upon contact. Please note that the nitrile or latex gloves that are used to collect saliva samples do not protect against dry ice burns. Additionally, there is a risk of asphyxiation from dry ice in areas with no ventilation as dry ice evaporates to the odorless carbon dioxide, so take caution when preparing saliva shipments.

Importantly, if shipping or driving your saliva samples to an independent lab for assay, familiarity with the local—and perhaps federal and international—laws surrounding the shipment of human body fluids and dry ice is of the utmost importance. Your institution's equivalent of an office of "environment, health, and safety" will be familiar with all the laws governing these shipments. Additionally, if you are shipping your samples across international borders, be sure to identify the forms you will need to ensure your samples are not detained or quarantined by customs officials.

Quantifying hormone levels. In order to analyze hormone data statistically, the hormones present in collected saliva samples have to be extracted and converted into numbers. The process of measuring hormone levels in human body fluids is called "assay." Many saliva-testing labs will assay saliva samples for a fee based on the number and type of hormone sampled or for a charge based on an hourly rate for labor.

Having highlighted the processes for collecting, storing, shipping, and assaying neuroendocrine measures, we now discuss important factors that should be considered when incorporating these measures into experimental designs.

Experimental Design Considerations

Incorporating neuroendocrine methods into an intergroup protocol requires little more than including time for saliva collection in the protocol. However, you will need to prescreen participants and ask them to refrain from certain prelab behaviors. The greatest impact that neuroendocrine methodologies will have on normal experimental design is that experimental tasks must be sufficiently high arousal to activate neuroendocrinological systems. Otherwise, designing intergroup studies that include hormones should be similar to designing intergroup studies without hormones.

Participant sampling. As with most research, it is important to sample as randomly as possible from the population of interest. However, with neuroendocrine measures, some restrictions on the participant sample and prelab behaviors will ensure the highest quality data.

Prescreening. One of the biggest considerations in neuroendocrine research is participant sex, because women's hormones change over the course of the menstrual cycle. Many researchers recruit women who are at the hormonal nadir of their menstrual cycle, which is the follicular stage that begins on the first day of menstruation and lasts approximately ten days. Prior to a lab visit, female participants are asked to report the first

day of their last period, and they are scheduled for a lab session that corresponds to their follicular stage. It should be noted, however, that if hormonal changes over the menstrual cycle itself is the research interest, then hormones measured across the menstrual cycle are comparable to each other.

Some studies exclude women taking hormonal birth control pills because birth control pills interfere with processes that suppress the HPA (Ansseau, Leboulle, Sulon, von Frenckell, & Legros, 1993). To prevent having a gender-biased sample, we prefer to address this challenge by using hormonal birth control as a covariate.

Additional exclusions include asking participants about chronic diseases relevant to the hormone of interest (e.g., Cushing's syndrome), as these diseases can overwhelm any variance in hormones explained by psychological factors.

Participant behavioral requirements. In order to maximize the psychological relevance of hormone measurement, behaviors that can interfere with hormone measurement should be controlled prior to a participant arriving at the lab. The two biggest threats to the integrity of a hormone sample that can come from participant behavior is blood in the sample and exogenous hormones or proteins contaminating the sample. Blood from bleeding gums due to flossing, gingivitis, or other dental ailments can contaminate the saliva sample, resulting in a pinkish color or sometimes even gum tissue floating in the sample. Samples with blood in them cannot be assayed and can be avoided by asking participants to not floss within 24 hours of coming to the lab and by screening out participants with a history of gingivitis. Participants should also be asked to refrain from eating dairy products prior to coming into the lab, especially dairy products that contain active cultures like yogurt. In general, it is best to ask people to not eat or drink anything for at least 2 hours before the start of the lab session, because this practice minimizes the chance that food or sugar get into the saliva sample.

Hormone sampling. Neuroendocrine methods involve repeated measurements of hormones,

and the timing of these measurements is fundamental to their interpretation. It is necessary to collect an initial "baseline" sample. After baseline, all other saliva samples are typically considered to be "reactivity" samples that are collected at set time intervals after key experimental events.

Baseline measurement. Most studies utilizing neuroendocrine measures will begin by collecting a snapshot of a participant's current physiological state upon arrival at the lab, which is referred to as their "baseline" or "tonic" measurement. Baseline measurements address two issues that would otherwise hinder physiological research. The Law of Initial Values (Wilder, 1962) posits that the magnitude of a physiological response is dependent on initial activity of that physiological system. In addition, the concept of individual stereotypy (Arena, Goldberg, Saul, & Hobbs, 1989) highlights individual differences in tonic values, such that it is difficult to compare single-point measurements of baseline hormones across individuals in a meaningful way. For these reasons, obtaining a good, clean baseline is paramount.

Hormonal reactivity. Almost as fundamental to the concept of baseline is the construct of reactivity. Reactivity is an event-based change in hormone levels from baseline. It is measured by collecting multiple saliva samples within an experimental session that are timed to correspond to key experimental events. Reactivity is most typically calculated as a difference score (e.g., subtracting the baseline value from subsequently collected values), although the standard issues with difference scores, such as poor reliability obscuring real effects, apply to hormone reactivity as they do to any other psychological measurement (Overall & Woodward, 1975). The two main alternatives for quantifying reactivity are to calculate "residualized" values or to simply conduct within-subjects analyses on raw hormone values. Residualized reactivity scores are calculated by regressing the reactivity sample on the baseline sample and using the residuals from this regression as the reactivity value. Some valid within-subjects analyses for examining

reactivity include paired-sample *t* tests, multilevel linear models, or mixed-effects ANOVA.

Sample timing. Hormone levels in saliva do not reflect the present values of that hormone circulating in the blood; they reflect levels at a previous time point. This timing is driven by the fact that hormones allow for "humoral" communication between cells through bodily fluids like saliva, yet this form of intracellular communication is slow relative to the communication speed of the nervous system. Therefore, to determine the hormonal response to an event, it is necessary to wait for a set time interval after the event before you collect saliva. When designing studies, record the timing of the key experimental events (e.g., the onset of the lab stressor occurs 25 minutes into the protocol) and then collect saliva samples after a certain time interval has passed (e.g., the sample that reflects reactions to the lab stressor is collected 45 minutes into the protocol). The correct time interval is specific to the hormone of interest (see Table 1).

Task specifications. An emphasis on reactivity necessitates a distinction between "active" and "passive" coping tasks (cf. Obrist, 1976). You should not expect a major change in hormones if your experimental task does not require the participant to act. While the central nervous system is highly reactive to stimuli, the peripheral nervous system's primary task is maintaining homeostasis. Activity in the peripheries will only be observed if participants are sufficiently engaged in the situation such that their body would have to change in some way to maintain homeostasis. It is for this reason that few neuroendocrine studies examine reactivity to video stimuli or to reaction time tasks, as both are passive tasks that require mental energy but not necessarily physical action (see Dickerson & Kemeny, 2004). In sum, to ensure adequate neuroendocrine reactivity, you should ensure that your experimental tasks are sufficiently engaging.

Altogether, incorporating hormones into existing intergroup paradigms is mostly a matter of leaving time for saliva collection. We recommend designing the psychological aspects of the study first and then adding in saliva collection to capture

Table 1. Timing of saliva samples relative to experimental events.

Neuroendocrine measure	Timing interval
Cortisol	15–20 minutes
DHEA	20 minutes
Alpha amylase	5 minutes
Testosterone	15–20 minutes
Oxytocin	12–20 minutes

Note. The neuroendocrine measures discussed in this paper are presented here along with information about how long you must wait to measure hormonal responses to study events, with ranges reflecting different recommendations in the literature. The timing interval should begin at the onset of the stressor.

baseline and reactivity values. As long as the experimental task requires participants to perform or engage in some form of action, then the foundation will be set for detecting meaningful changes in hormones.

Biosafety Certification

In many jurisdictions, biohazard laws regulate the collection, handling, and storage of human body fluids. Most jurisdictions view saliva samples as potentially infectious diseases. Indeed, in any given sample you collect, it is likely that at least one participant will be sick without knowing that they are, and the saliva you collect from them has the potential to spread the disease after it has been separated from its carrier. Most universities will have a biosafety office, and you should consult with this office prior to initiating saliva collection in your lab to ensure conformity to local laws. You may need to obtain certification from this office before you are allowed to collect or store human body fluids. Most likely, you and all members of your research team will have to attend a biosafety training course that teaches safe lab practices.

Specific Hormones and Considerations for Assay and Analysis

A number of the hormones described earlier in this paper can be measured in various ways and

have specifications that should be considered in advance of collection. We begin with specifics regarding one of the most commonly studied hormones, cortisol, and end with a discussion about measuring the peptide oxytocin.

Cortisol measures. Cortisol exhibits a diurnal cycle that is triggered by fluctuations in light over the course of the day. Cortisol levels peak approximately 30 minutes after waking and steadily fall during the daylight hours. Cortisol levels begin to rise again at nighttime during sleep. The nadir of the diurnal cycle of cortisol occurs in the early evening, although circulating cortisol levels are low enough to detect changes due to psychological stimuli by the afternoon. This diurnal cycle has implications for when cortisol should be collected in intergroup research (i.e., during afternoon hours) to ensure that the variance that is accounted for by these natural processes does not drown out the psychological variance in cortisol that can be attributed to intergroup processes.

In addition to collecting cortisol via saliva, cortisol can also be collected through hair samples, a methodology we describe below. Additionally, CAR measurement requires the use of specific analytic tools described in this section.

Hair cortisol. Within the last decade, researchers have begun to measure the concentrations of cortisol in hair to capture regular activation of the HPA. Humans who should have greater systemic exposure to cortisol for biological reason, such as pregnant women in the third trimester (Kirschbaum, Tietze, Skoluda, & Dettenborn, 2009) and patients with Cushing's syndrome (Thomson et al., 2010), show elevated levels of cortisol in their hair. From a psychosocial perspective, elevated hair cortisol levels has been found among individuals experiencing chronic stress, either through long-term unemployment (Dettenborn, Tietze, Bruckner, & Kirschbaum, 2010) or chronic pain (van Uum et al., 2008). Thus, it appears that hair cortisol levels indeed reflect systemic exposure to cortisol over time.

Hair cortisol is relatively easy to collect. Hair grows at a rate of approximately 1 cm per month,

so the amount of hair that you need to collect depends on the time period you want to study. Typically, screening requirements for hair cortisol studies require participants to have at least 3 cm of hair, making this collection method more prevalent among female participant populations.

Participants are asked to provide a 1 cm² sample of hair from the back of their head that is cut at the scalp using sterilized scissors. After obtaining the tuft of hair, the sample must be laid on a piece of tinfoil, which is then folded and enclosed around the sample without bending the hair. A permanent marker is used to draw on the tinfoil to indicate which side of the sample was closest to the scalp (i.e., the follicle side). Finally, the tinfoil should be placed in an envelope and labeled with the participant ID number. Depending on the humidity of the local environment, hair samples may need to be stored under some form of climate control (e.g., a humidor) until they can be shipped to an independent lab for assay. Depending on the amount of hair you collected from participants, it is also possible to assay the hair samples in 1-cm segments, which will reflect changes in HPA exposure over time (e.g., Kirschbaum et al., 2009).

CAR. As mentioned before, the CAR occurs in the first hour of waking, so it is measured through repeated saliva sampling during this hour. Typically, the CAR is measured by collecting four saliva samples at 0, 30, 45, and 60 minutes after awakening, although some researchers choose to either collect an additional sample at 15 minutes after waking or to forgo the 45-minute sample. Since timing is critical, the CAR is typically measured in participants' homes. Participants first attend a training session, where the importance of sample timing is strongly emphasized. They are sent home with prelabeled cryovials and a storage box to keep the samples. Many researchers use technology such as web surveys or text messages to help participants comply with the instructions. After they have collected the samples, the researchers arrange a time to meet the participant and exchange the samples. Cortisol may be stored at room temperature for at

least 7 days before freezing (Gröschl et al., 2001; Kirschbaum & Hellhammer, 2000).

The traditional approach to statistical analysis of the CAR is to combine these four samples into one metric by calculating the area under the curve (Pruessner, Kirschbaum, Meinlschmid, & Hellhammer, 2003). However, this method reflects the total volume of cortisol released instead of the morphology of the CAR waveform, which is one of the key reasons to collect CAR. Moreover, it is unclear whether a greater volume of cortisol should be expected among people with well-regulated systems (i.e., because they had the largest spike of cortisol at 30 minutes after waking) or among people with dysregulated systems (i.e., because they did not show as steep of a decline between 30 to 60 minutes). As a result, we recommend that researchers interested in the CAR use mixed models or multilevel models with polynomial effects of time (e.g., Cortisol = Time + Time^2) to more directly capture the curvilinear essence of the CAR.

Oxytocin. It is important to note that there is controversy surrounding the assay and administration of oxytocin. Oxytocin assays using commercial kits have undergone considerable scrutiny in the last year. A recent review (McCullough, Churchland, & Mendez, 2013) demonstrated that assays performed without first extracting other proteins from the sample yield levels of oxytocin almost two orders of magnitude higher than values obtained after conducting the preliminary protein extraction, but that this method is nonetheless increasing in popularity due to its relatively economical price. Moreover, the proteins that could interfere with the oxytocin assay are uncorrelated with oxytocin levels, thus it is virtually impossible to assess how much of the measured "oxytocin" from an assay conducted without extraction was actually oxytocin.

Summary

Altogether, salivary methods provide intergroup researchers with a flexible way to incorporate physiological constructs into their research questions. Setting up a neuroendocrine lab involves obtaining lab biosafety certification, purchasing disposable test tubes and gloves, and gaining access to a freezer. After this initial startup, adding hormones to new study protocols is relatively smooth. All the same, each neuroendocrine measure has its own unique considerations, and the method and context of saliva collection will shape the interpretation of results. Readers who would like to learn more about neuroendocrinological theory and methods are referred to the cellular and humoral systems section of the *Handbook of Psychophysiology* (Cacioppo, Tassinary, & Berntson, 2007), with a specific emphasis on the chapters covering the neuroendocrine stress systems (Kaltsas & Chrousos, 2007), reproductive hormones (Snowdon & Ziegler, 2007), and immune responses (Dhabhar, 2007).

Psychophysiological Inference

As previously stated, neuroendocrine responses cannot be controlled consciously, and thus are particularly suited to research in intergroup relations. However, the field of psychophysiology has not advanced to the point where we can identify psychological states by observing physiological responses alone. Relationships between psychological and physiological states are multiplicitous and frequently context-dependent. As such, Cacioppo and Tassinary (1990) laid out a taxonomy for inferences about the relationship between the psychological and physiological domains. In short, three core dimensions can be used to classify psychophysiological relationships: specificity, generality, and sensitivity. The specificity dimension ranges from a one-to-one relationship between a particular psychological construct and a particular physiological measure to a many-to-one relationship between multiple psychological constructs and a particular physiological measure. Most neuroendocrine measures have been correlated with multiple psychological constructs. The implications of a psychophysiological relationship having a one-to-one specificity are that the physiological state is always present

when the psychological state is present and the psychological state is always present when the physiological state is present. The only way to prove a one-to-one relationship is to do many studies where both the psychological and physiological states are experimentally manipulated. The generality dimension ranges from context-dependent to context-free. When a new association between constructs in the psychological and physiological domains is identified, we begin by assuming that the relationship is context-dependent. It is only after the relationship has been shown to exist across different tasks, independent labs, and various geographical locations would we consider a psychophysiological relationship to be context-free. Finally, the sensitivity dimension refers to whether a particular physiological measure has sufficient variance explained by psychological events to reliably capture and predict it.

The specificity and generality dimensions are combined to create four categories of psychophysiological relationships (Cacioppo & Tassinary, 1990). At the many-to-one end of the specificity dimension, a psychophysiological relationship will be called an "outcome" if it is context-dependent and a "concomitant" if it is context-free. At the one-to-one end of the specificity dimension, a psychophysiological relationship will be called a "marker" if it is context-dependent and an "index" if it is context-free. These terms should be used with rigor and precision, because they help to clarify our current understanding of any given psychophysiological relationship.

Future of Intergroup Neuroendocrinology

As an interdisciplinary subject area, intergroup neuroendocrinology benefits from the extensive research that comprises the intergroup relations and neuroendocrine literatures. All the same, there is a relative paucity of neuroendocrine methods in research on intergroup relations, relative to self-report and behavioral measures. Although hormones are relatively free from the impact of socially desirable responding, the potential value of incorporating neuroendocrine measures into intergroup studies goes far beyond tapping into a measure outside of conscious control. The fields of biological psychology, endocrinology, physiology, and medicine provide a multifaceted lens through which we can understand the meaning of hormonal responses for intergroup processes; charting the physiological patterns that covary with intergroup processes is the first step towards bridging these knowledge bases.

A major outstanding question in intergroup neuroendocrinology is the degree to which hormonal responses feed back to affect social experiences. Intergroup research involving hormones has primarily looked at hormonal responses to intergroup situations (e.g., Amodio, 2009; Page-Gould et al., 2008). As a result of primarily conceptualizing hormones as outcome variables, we know much more about how the body reacts to intergroup processes than we do about how intergroup processes react to the body. The primary hurdle to examining the impact of hormonal processes on psychological experiences is the invasiveness involved in experimentally manipulating hormones, but noninvasive methods for manipulating hormone levels do exist. For example, Akinola and Mendes (2012) examined the impact of cortisol reactivity on racially biased responding in a reaction time task by experimentally manipulating activation of the HPA axis through a laboratory stressor. We expect that people are largely unaware of their physiological responses, but that individual differences likely exist in the degree to which hormones relate to subjective experience (e.g., Adam et al., 2006).

As statistical methods become increasingly able to handle complex data structures (e.g., multilevel modeling, structural equation modeling, Bayesian models), we anticipate that factors that were once considered nuisance variables in hormonal research will become the focus of study. For example, although many psychoneuroendocrinologists restrict female samples to the hormonal nadir of their menstrual cycle, this design choice removes the opportunity to observe variance that can be

systematically attributed to menstrual cycle (e.g., Gangestad, Thornhill, & Garver-Apgar, 2010; Salvatore, Meltzer, Gaertner, under review). Similarly, instead of using certain variables such as waking time and birth control as covariates, researchers may consider whether these physiological factors moderate psychophysiological relationships between intergroup processes and hormones in systematic and meaningful ways (e.g., Alvergne & Lummaa, 2010).

Conclusions

Both intergroup relations and neuroendocrinology are fascinating fields that can learn from each other. As advances continue to be made in biological measurement and statistics, we are hopeful that researchers will capitalize on more integrated, interdisciplinary approaches in psychology. Using neuroendocrine measures in intergroup research and incorporating multiple levels of phenomenological experience into intergroup theories and observations will deepen our understanding of intergroup processes and the ways that basic biological processes respond to, enhance, and inhibit intergroup relations. Our hope is that this paper will serve to embolden intergroup researchers to embrace neuroendocrine methods with the genuine fervor that is only satiated by using these measures with empirical and theoretical rigor.

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