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PSYCHOBIOLOGICAL MEASUREMENT

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Introduction

There has been a longstanding interest in mind-body phenomenon in psychology. Indeed, psychologist William James' (1884) influential theory on emotion unequivocally linked physiological changes to emotions. Although his theory has been modified in the many ensuing years, James' work was among the first to suggest that measuring patterns of arousal would allow psychologists to index emotional states. Nearly a century later, multiple texts on psychophysiological methods are available for students and researchers. Together with many technological advances in understanding and quantifying human anatomy and physiology in more nuanced ways, we now see bourgeoning interest and rigorous psychobiological research among social scientists.

Some social scientists look to psychobiological assessment as objective measures to replace or complement self-report measures. Just as implicit or behavioral observations may be used to circumvent the limitations of introspection or self-reported psychological processes, so is the hope for biological measures. The collection of biological data alongside self-report or behavioral measures may confirm participant reports or provide converging evidence of a particular phenomenon, such as arousal or threat perception. Researchers might also turn to biological measurement to identify underlying mechanisms that contribute to psychological processes. For instance, one might test whether specific hormone secretions precede particular social behaviors or psychological phenomenon. Still others might focus on biological measures as consequences of psychological or behavioral phenomenon. For example, does psychosocial stress lead to greater susceptibility to the common cold?

Although all such approaches in the context of psychobiological assessment are addressed in this chapter, it is important to note that psychobiological data are often collected in a way that makes it difficult to tease apart cause and effect. In some instances, it seems clear that psychological processes are driving physiological changes; other times physiological processes seem to be leading to psychological responses. For those who turn to psychobiological assessment for alternatives to self-report or behavioral data, the cause and effect may not matter so much, as long as the presence or absence of a correlation can be determined. For others, clearly identifying cause and consequence is critical for confirming and refining theory.

This chapter begins by providing basic anatomical and physiological information about each of three pertinent bodily systems: autonomic, endocrine, and immune. Along with each system overview are procedures for assessing specific system parameters or biomarkers and select examples of research to illustrate topics that can be addressed with such methods. The chapter then reviews general methodological, practical, and analytical issues one should consider and closes with concluding remarks, sharing thoughts on next steps for this research area, and by providing additional resources for the reader.

Autonomic Nervous System

Anatomy and Physiology

The autonomic nervous system (ANS) is a major division of the peripheral nervous system. It regulates critical peripheral bodily functions that are generally thought to be outside of our conscious control. ANS nerve fibers transmit signals between the brain and spinal cord and nearly every other tissue and vessel throughout the body's periphery, including smooth and cardiac muscle, as well as glands, and the gastrointestinal track. Blood pressure, heart rate, digestion, body temperature, respiration, and inflammation are just some of the many functions under ANS control (Andreassi, 2007).

The ANS is further divided into two functionally distinct branches: the parasympathetic nervous system (PNS) and the sympathetic nervous system (SNS). Broadly speaking, SNS activity facilitates bodily processes associated with energy mobilization, such as those that help an individual deal with emergency, or "fight-or-flight" types of situations. Consider how your body might respond if a fire alarm goes off in your building. As described by physiologist Walter Cannon (1915), one might experience

> the contraction of blood vessels with resulting pallor, the pouring out of "cold sweat," the stopping of saliva-flow so that "the tongue cleaves to the roof of the mouth," the dilation of pupils, the rising of the hairs, the rapid

> beating of the heart, the hurried respiration, the trembling and twitching of the muscles.
>
> *(p. 3)*

Such cardinal features of sympathetic arousal divert blood flow and energy to major muscles throughout the body, and are understood to enhance survival of physical threats to the self.

The other branch of the ANS, the PNS, exerts actions in opposition to the SNS and leads to vegetative and restorative functions. As such, PNS activity is sometimes characterized as "rest-and-digest" or "feed-and-breed," to exemplify some of the functions this system serves. Consider how your body might respond after you finish your extra helping on Thanksgiving and you are about to settle in for the evening. The PNS stimulates the digestive system (salivary flow, peristalsis in the intestines), and it promotes rest, relaxation, and sleep. PNS neurons also innervate the smooth muscles of the iris and lead to constriction of the pupil. A particularly important cranial nerve of the ANS, the vagus nerve, transmits signals between the brain and periphery, including control of the heart. When activated, it slows the beating of the heart. Of particular relevance to those interested in psychobiological measures, the vagus has both "afferent" and "efferent" pathways. In other words, because messages flow both from the central nervous system to peripheral organs (afferent) and away from peripheral organs to the central nervous system (efferent), the body and brain can influence one another as well as coordinate a response to external changes in the physical and social environment.

Combined, the SNS and PNS are integrated subsystems that together facilitate homeostasis, or the underlying processes of maintaining a relatively stable internal environment essential for survival. As integrative systems, the two can at times promote the same responses, through opposing mechanisms. For instance, signs of "fight-or-flight" activity, such as increased beating of the heart, may be influenced by both stimulation of the SNS and a withdrawal of PNS activity. As a result, there has been some interest in determining the balance of ANS activity, or the degree to which one ANS branch is more activated than the other. To properly assess the complex, interdependent aspects of physiological systems it is necessary to use strong measurement tools—the topic of this chapter.

Assessment

A variety of parameters are used to quantify SNS and PNS activity. Peripheral ANS parameters are derived most commonly from observed cardiovascular and electrodermal activity. See Table 4.1 for a summary of ANS indices in social psychological research. As noted earlier, the SNS and PNS systems work in conjunction with one another, and each parameter varies in the degree to which it reflects mostly SNS activity, PNS, or both.

TABLE 4.1 Autonomic Nervous System Indices in Social Psychological Research

Parameter	*Description*	*Sympathetic Response*	*Parasympathetic Response*	*Instrumentation*
Cardiovascular				
HR	Speed or rate of heart contractions in beats per minute.	Increase	Decrease	Electrocardiography, pulse meter
SBP	Systolic blood pressure occurs when the heart contracts (i.e., maximum pressure in artery) in millimeters of mercury.	Increase	Decrease	Blood pressure monitor
DBP	Diastolic blood pressure occurs in between contractions of the heart (i.e., minimum pressure in artery) in millimeters of mercury.	Increase	Decrease	Blood pressure monitor
MAP	Mean arterial pressure, typically derived with the following formula: 1/3(SBP-DBP) + DBP = MAP.	Increase	Decrease	Blood pressure monitor
CO	Cardiac output reflects amount of blood pumped by the heart in liters per minute (HR × stroke volume).	Increase	Decrease	Impedance cardiography and electrocardiography
TPR	Total peripheral resistance, or net constriction in the arterial system (MAP × 80 / CO) in resistance units.	Increase	Decrease	Impedance cardiography and electrocardiography
PEP	Pre-ejection period is the amount of time between depolarization in the left ventricle and the opening of the aortic valve in milliseconds. PEP is also referred to as ventricular contractility.	Decrease		Impedance cardiography and electrocardiography
RMSSD	Root mean square of successive differences is a time-domain measure of variability in heart rate measured in milliseconds. RMSSD is calculated by taking the square root of the mean squared difference of successive normal-to-normal heart beats over a specified time period.		Increase	Electrocardiography

RSA or HF-HRV	Respiratory sinus arrhythmia, or high frequency HR variability, refers to the beat-to-beat alterations in heart rate occurring in the high frequency range (0.15-0.40 Hz) as measured by power spectral analysis. HF-HRV is often reported in squared millisecond units.		Increase	Electrocardiography, select pulse meters
Electrodermal				
SCL	Skin conductance level is the tonic level of the skin's electrical conductivity in microSiemens.	Increase		Electrodermal activity recording system
SCR	Skin conductance response is the phasic change in skin's electrical conductivity in response to a stimulus in micro-Siemens.	Increase		Electrodermal activity recording system
NS-SCR	Non-specific skin conductance response is the phasic change in skin's electrical conductivity that occurs in the absence of an identifiable stimulus in microSiemens.	Increase		Electrodermal activity recording system
Other				
RR	Respiratory rate is the number of breaths per minute.	Increase	Decrease	Respiratory belt
Pupil size	Diameter of pupil, measured in millimeters.	Decrease	Increase	Eye-tracking system

Cardiovascular indices

Heart rate, or the number of times the ventricles of the heart contract in a given period of time (usually a minute), has been widely used to capture ANS activity in psychological research. For example, many researchers have quantified phasic changes (i.e., pre-post increases) in HR to index psychological stress induced in the laboratory. One reason for HR's popularity is likely due at least in part to its relative ease of assessment. You can't get much more low-tech than simply placing two fingers on a wrist to count pulse rate! However, since both sympathetic and parasympathetic inputs control the rate and variability of HR (Saul, 1990), researchers have turned more recently to examine SNS and PNS activity separately.

Beyond HR, additional cardiac parameters allow for a more nuanced assessment of ANS activity (see Table 4.1). For example, high frequency HR reflects cardiac input from the vagus nerve, and thus can be used as an index of PNS activity. HR variability measures reflect the degree to which intervals between ventricular contractions vary. Variability in HR can be calculated in a multiple of ways (for review, see Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996).

Electrocardiography (ECG or EKG) and impedance cardiography are commonly used to derive ANS measures. ECG recordings capture multiple electrical inflections of the heart's electrical activity (e.g., QRS complexes), which can later be used to derive multiple cardiac parameters. Impedance cardiography recordings capture changes in blood flow to estimate volume of blood ejected from the heart and the timing of the opening and closing of the heart's valves. In a typical psychophysiology research laboratory, a three-lead ECG with self-adhering disposable spot electrodes are placed on a participant to acquire the cardiac signal. Electrodes are typically placed on the limbs or torso to create an imaginary triangle with the heart at the center (Andreassi, 2007). The exact configuration of the electrodes can vary somewhat to accommodate particular study constraints. For example, if movement of the limbs is anticipated during the laboratory procedures, the researcher may choose to place the leads on the torso. To acquire impedance data alongside ECG, additional spot or band/tape electrodes are necessary. With band electrodes, two may be placed to encircle the base of the neck and an additional two are placed near and below the base of the sternum to encircle the torso (for additional details, see Blascovitch, Vanman, Mendes, & Dickerson, 2011).

If you have been keeping count, we are now up to seven electrodes placed on the participant—with just as many cables fastened to them! Such tethering is important to take into account when designing study protocols and providing pre-visit information to participants. For instance, it would be a good idea to instruct participants to wear comfortable, two-piece clothing, leave their jewelry at home, and use the restroom before being hooked up to the physiological recording equipment.

Although wired recording systems remain normative, commercially available ambulatory devices can be used inside and outside the research laboratory. Indeed, modestly priced ambulatory HR monitoring devices have shown to have excellent test-retest reliability and agreement with ECG-derived HR variability in healthy participants (Weippert et al., 2010; Williams et al., 2016; for ambulatory cardiac measures, see Walsh, Topol, & Steinhubl, 2014). Fully equipped psychophysiological systems that amplify and record ANS-related activity can cost a few to tens of thousands of dollars, so purchasing decisions are not to be taken lightly. Consider the applications you and your collaborators may want now and in many years from the time of the purchase. Smaller systems or some ambulatory devices with more limited assessment options can be much lower in cost. Ambulatory devices worn in the laboratory might also be desirable given that traditional systems tether participants to equipment with multiple sensors and cords. It is always a good idea to consult with other researchers who have used the equipment under your consideration or to try it out yourself before making a large financial commitment.

Electrodermal indices

Electrodermal activity refers to all electrical phenomena in the skin. Of most relevance to the assessment of the ANS, is the electrical activity of eccrine sweat glands. Eccrine sweat glands are innervated by cholinergic fibers of the SNS, and thus electrodermal activity is commonly used to quantify sympathetic activity. Although the primary function of sweat glands are to regulate body temperature, or thermoregulation, they are also responsive to psychologically relevant stimuli. Some of the early electrodermal activity work focused "arousal," which was quantified by using a galvanometer to measure changes in skin conductance in response to a variety of emotional stimuli (Neumann & Blanton, 1970).

There are two main types of methods for collecting electrodermal activity with commercially available instruments and software (Andreassi, 2007): skin conductance and skin potential. For skin conductance, a small electrical current is passed through the skin with two (bipolar) electrodes placed on the skin surface. The resistance to that current, or its reciprocal skin conductance, is measured. Skin conductance levels (SCL) refer to "baseline" values at a given time period. Skin conductance/resistance responses refer to momentary fluctuations, typically in response to a stimuli introduced by the research. The skin conductance response (SCR) typically appears one to three seconds after a stimulus is presented. It is important to note that SCRs also occur spontaneously, or in the absence of the researcher's stimulus. Such a response is referred to as a "non-specific" SCR (NS-SCR). Researchers will commonly measure the number of NS-SCRs in a period of time (i.e., 1 minute) and examine whether the NS-SCR rate changes in response to particular stimuli of interest. In the second type of electrodermal activity assessment (skin potential), no electrical current is introduced. Instead,

one (unipolar) electrode is placed on the skin to measure skin potential at a given point in time.

Eccrine sweat glands are found widely throughout the surface of the body, and are most densely distributed across the palms of hands and soles of feet. Thus, typical electrode placement for skin conductance is on two adjacent fingers or palm of one hand with a reference electrode placed on an abraded forearm. For skin potential assessment, one electrode is placed on the palm and a reference electrode is placed on an abraded forearm. Often researchers will place electrodes on the non-dominant hand and arm so as to limit interference with participant movement. Though this may make good practical sense, the reader is cautioned that electrodermal activity may differ from one side of the body of the other. Indeed, some psychophysiologists have specifically focused on electrodermal activity asymmetry in their research (e.g., Picard, Fedor, & Ayzenberg, 2016).

Just as with cardiac activity recording devices, there are stationary and portable devices for electrodermal activity (e.g., Poh, Swenson, & Picard, 2010). Although there are many advantages to being able to record electrodermal activity wirelessly and in daily life settings, it is important to note that electrodermal activity recorded from other skin locations (i.e., wrists) may differ from what has been found for palms, and may be smaller in magnitude (Payne, Schell, & Dawson, 2016).

Example Research Applications

Social psychological research applications with ANS measurement are extensive—ranging from simple measures of arousal in early cognitive dissonance studies to more recent and complex assessment of parasympathetic and sympathetic balance in response to stressful stimuli. One of the most common social psychological research applications is in the area of emotion. Although a thorough review of ANS-emotion findings are beyond the scope of this chapter (see, Kriebig, 2010; Levenson, 2014), it is fair to say that the use of emotion elicitation paradigms in modern psychophysiological laboratories has led to a variety of insights on the links between emotion, emotion regulation, and ANS activity. For example, HR variability has emerged as a potential index of regulated emotional responding (Appelhans & Luecken, 2006). Just as emotion regulation reflects one's ability to adjust one's arousal and emotional state in response to changing emotional stimuli, HR variability reflects the degree to which the beating of one's heart can be altered to meet the demands of the changing environment (with higher HR variability indicating greater ability to transition between arousal levels). Accordingly, higher resting HR variability has been linked to a number of variables that indicate regulated emotional responding (e.g., constructive coping, faster fear extinction). Other ANS indices have been shown to correlate with other social psychological states and processes, such as empathy, social support, and stress appraisals. See Table 4.2 for more examples of social psychological research utilizing ANS indices.

TABLE 4.2 Example Social Psychological Research Applications Utilizing Autonomic Nervous System (ANS) Indices

Topic	*ANS Parameter(s)*	*Citation*	*Description*
Emotion	*many*	Kreibig, 2010	This review demonstrated some patterns in ANS responses to emotion subtypes and discrete emotions.
Emotion regulation	HRV	Applehans & Luecken, 2006	This review indicated that resting HRV is associated with less negativity bias, increased approach to novelty, constructive coping, and faster fear extinction.
Empathy	SC	Levenson & Ruef, 1992	Participants in their study who more accurately rated negative affect experienced by another had a more similar SC response to that person.
Social support	HR, BP, SC	Thorsteinsson & James, 1999	This meta-analysis showed that experimental manipulations of social support provision have moderate to large effects on HR and BP reactivity.
Stress appraisal	CO, TPR, PEP, HR	Seery, 2011	This review demonstrated that in response to self-relevant motivated performance situations, challenge states are characterized by relatively greater CO and lower TPR and threat states are characterized by relatively lower CO and higher TPR.

Endocrine System

Anatomy and Physiology

Similar to the nervous system, the endocrine system functions as a major communication system that consists of glands and hormones, the chemical messengers that glands secrete. Although the glands and organs of the endocrine system are widely distributed across the body and not anatomically continuous, they are tied together as a system based on the functions they serve. Furthermore, the nervous system is well integrated with the endocrine system, which underlies the close connections between psychological processes and hormonal changes. The study of hormones and hormonal processes in relation to psychological processes

and human behavior is referred to as psychoneuroendocrinology or behavioral endocrinology.

There are three classes of hormones, which control and regulate the activity of cells and organs (Neave, 2008). The majority are protein-based (peptide hormones), but others are derived from amino acids (amine hormones) or lipids (steroid hormones). See Table 4.3 for a list of hormones by class, their source, and their primary effects. The chemical structure of a hormone, or class, is important to note because it influences the transportation, movement, and half-life of hormones, and thus their assessment. For instance, steroid hormones, such as cortisol, are derived from cholesterol and can pass through cell walls and thus enter most

TABLE 4.3 Major Hormones and Their Primary Effects

Hormone	*Source*	*Primary Effects*
Peptide Hormones		
Adrenocorticotropin hormone	Anterior pituitary	Release of glucocorticoids and androgens
Corticotropin-releasing hormone	Hypothalamus	Release of adrenocorticotropin hormone
Gastrin	Stomach	Stomach acid secretion
Glucagon	Pancreas	Increase in blood sugar
Growth hormone	Anterior pituitary	Body growth and metabolic processes
Insulin	Pancreas	Reduction in blood sugar, promotion of cellular uptake of glucose, formation of glycogen
Leptin	Adipose tissue	Appetite suppression
Oxytocin	Hypothalamus (via posterior pituitary)	Contraction of uterus and mammary glands
Prolactin	Anterior pituitary	Lactation
Vasopressin	Hypothalamus (via posterior pituitary)	Vasoconstriction, water retention
Amine Hormones		
Epinephrine (or adrenaline)	Adrenal medulla	Preparation for emergency: increase in blood sugar, vasoconstriction, HR and BP
Melatonin	Pineal gland	Regulation of circadian timing
Norepinephrine (or noradrenaline)	Adrenal medulla	Preparation for emergency: increase in blood sugar, vasoconstriction, HR and BP

Hormone	*Source*	*Primary Effects*
Thyroxine	Thyroid	Stimulation and regulation of metabolism
Steroid hormones		
Cortisol	Adrenal cortex	Help body cope for moderate/long-term stress: increase in blood sugar; immune function alteration
Dehydroepiandrosterone	Adrenal cortex	Serves as a precursor to gonadal hormones and to oppose effects of glucocorticoids
Estrogens (estradiol, estrone, estriol)	Ovaries	Regulation of menstrual cycle and development of female reproductive system
Progesterone	Ovaries	Regulation of menstrual cycle and development of female reproductive system
Testosterone	Testes	Sperm production, growth and development of male reproductive system

tissues throughout the body to bind to receptors. Steroid hormones also easily cross the blood-brain barrier to exert effects both on the brain and periphery. In contrast, protein-based hormones, such as the neuropeptide oxytocin, bind to surface receptors and activate secondary messenger systems to exert their effects.

Two endocrine glands of major significance include the hypothalamus and pituitary. Located near the center of the brain, the hypothalamus is made up of more than two dozen nuclei and serves as a "control" center, linking the nervous and endocrine systems. It plays a vital role in maintaining homeostatic processes, such as those described in the earlier section on the autonomic nervous system. The hypothalamus largely exerts its regulating effects by controlling the release of hormones from the pituitary gland, which is suspended off of the hypothalamus. Often referred to as the "master gland," the pituitary gland regulates many bodily functions, including growth, pain, blood pressure, and reproduction. Technically, the pituitary is two glands: the posterior pituitary and the anterior pituitary. The posterior pituitary stores and releases a variety of hormones that are produced by the hypothalamic nuclei (e.g., oxytocin, vasopressin) upon neural input from the central nervous system. The anterior pituitary has no neural inputs. Instead, other hormones trigger the anterior pituitary to synthesize and release additional hormones.

Hormones are typically released in a pulsatile fashion, meaning that glands secrete hormones multiple times throughout the day in brief surges. As such, the concentrations of hormones can fluctuate minute to minute. The secretion of a

given hormone is controlled by the concentrations of another, which is referred to as a hormone or biological cascade. Although there are several biological cascades within the endocrine system, the present focus is on one of the most prominent: the hypothalamic-pituitary-adrenal (HPA) axis and its end-product cortisol.

The HPA axis is activated by the hypothalamus in the brain, which receives and integrates somatosensory and affective input from the prefrontal cortex and limbic structures. In response, corticotrophin releasing hormone (CRH) is secreted by the neurons of the hypothalamus' paraventricular nucleus. CRH in turn triggers the release of adrenocorticotropin hormone (ACTH) from the anterior pituitary. ACTH stimulates the adrenal cortex to release glucocorticoids, including the catabolic steroid hormone cortisol. Biologically active cortisol acts independently on receptors distributed throughout the body and in conjunction with other physiological systems to regulate many functions critical for survival. For instance, cortisol acts with catecholamines of the autonomic nervous system to mobilize energy stores in response to stressors and also suppresses some aspects of the immune system and can inhibit reproductive processes. The HPA axis is regulated by negative feedback loops; as such, activation of the HPA axis is suppressed in response to elevated cortisol concentrations in healthy individuals. There is a pronounced circadian influence on cortisol concentrations; healthy individuals typically exhibit a robust increase in cortisol within the first 30–45 minutes after awakening, followed by decline across the rest of the day.

Assessment

Cortisol

Cortisol levels can be quantified from multiple biological specimens, most frequently from saliva and to a lesser extent blood. By collecting multiple blood or saliva samples, a researcher can document fluctuations in cortisol concentration over relatively short time periods (minutes to hours). Under "baseline" or resting conditions, approximately 5%–10% of circulating cortisol in the blood is free unbound, active), while the remaining is bound to proteins (corticosteroid binding globulin, serum albumin). Cortisol derived from blood represents both bound and unbound cortisol, with the latter reflecting the portion that is bioavailable, or free to act on target cells. Salivary concentrations, in contrast, reflect all bioavailable cortisol.

The sample of choice for most social scientists is saliva as it represents the freely available portion of cortisol, is non-invasive to collect, and easy to handle and store (relative to urine or blood), which allows for both laboratory- and field-based research. Saliva samples can be collected by passively drooling into a plastic tube via a straw or straw-like device or by using a commercially available device,

such as the Salivette (Sarstedt, Inc., Newton, NC), which includes a dental roll that participants put in mouths and saturate with saliva. It is good practice to store saliva samples in a freezer as soon as possible after collection until they are ready to be processed in-batch. However, it bears noting that cortisol levels are stable at room temperature for several weeks (Clements & Parker, 1998). Competitive enzyme linked immunosorbant assay determines how much of a substance (cortisol) is present in a sample.

Short-term changes in salivary cortisol concentration are commonly assessed in one of two general ways: (a) daily trajectories or slopes, and (b) responses to stressful events or stimuli, such as in the laboratory or in everyday life. For researchers interested in quantifying longer-term exposure to cortisol (months), hair can be a useful specimen to collect (Russell, Koren, Rieder, & Van Uum, 2012).

Multiple guidelines and recommendations have already been published for the collection of cortisol (Granger et al., 2007; Kudielka, Gierens, Hellhammer, Wüst, & Schlotz, 2012). However, a few basic recommendations are worth repeating here. First, sampling time points should be standardized across participants and the actual times of collection should be recorded. This is important because of the dramatic diurnal fluctuation in cortisol concentrations. Second, medical histories (e.g., endocrine and psychiatric disorders), medication use (e.g., oral contraception), and lifestyle factors (smoking, physical activity) can shape resting and reactive cortisol levels. As such, researchers typically exclude from participation or analyses cases in which participants use steroid-based medication, are pregnant, or have a major psychiatric or endocrine disorder. Depending on a particular study, additional inclusion or exclusion criteria might be applied.

Other hormones

Many of the same recommendation are in place for the assessment of other steroid hormones, such as testosterone or estradiol. For example, as with cortisol, circadian variation is commonly observed for other hormones. For ovarian sex hormones (e.g., progesterone, estradiol), phase of menstrual cycle can also dramatically alter hormone concentrations. To address this, some researchers schedule premenopausal women participants based on the day or phase of their menstrual cycle or control for it in analyses. It is also important to account for sex differences in research on steroid hormones, such as testosterone or estrogens. Passive drool is the recommend method for collecting saliva for quantifying most steroid hormones as cotton swabs can interfere with immunoassays (Granger et al., 2007). Although circulating peptide and amine hormones tonic and phasic levels can be readily quantified from blood samples, they are not reliably quantified in saliva. Additionally, large peptide hormones such as oxytocin do not cross the blood-brain barrier in significant amounts; therefore, peripheral blood concentrations may not reflect central levels found in the brain (for review, see Leng & Ludwig, 2016).

Example Research Applications

Burgeoning research of human psychoneuroendocrinology and social endocrinology focus on the bidirectional influences of social psychological processes and endocrine activity. Psychoneuroendocrinology researchers ask: can internal hormone changes influence psychosocial processes and can psychological and behavioral processes influence hormonal states? One of the most well-developed research topics in this area is psychological stress. Although conceptual and operational definitions of stress vary substantially between researchers, there is robust evidence that psychological stressors and perceptions of stress can have wide ranging effects on circulating hormones. Thus, many researchers incorporate endocrine assessment (e.g., salivary cortisol) to index psychological stress.

Additional psychoneuroendocrine research focuses on the links between testosterone and psychosocial processes such as aggression and dominance. Recent theoretical and empirical work incorporates both the catabolic hormone of cortisol and the anabolic hormone of testosterone to understand status, dominance, and testosterone. According to the dual-hormone hypothesis (Mehta & Josephs, 2010), testosterone is expected to influence aggressive and dominant behaviors when cortisol is low, but not when cortisol levels are high because cortisol counteracts the effects of testosterone. Consistent with this account, several studies have linked a hormone profile of high testosterone and low cortisol with increased dominance (e.g., Sherman, Lerner, Josephs, Renshon, & Gross, 2016). For more examples of other psychoneuroendocrine topics, including memory and prosocial behavior, see Table 4.4.

Immune System

Anatomy and Physiology

Immunity refers to the body's ability to resist or eliminate potentially harmful foreign materials or abnormal cells, and the cells and molecules of the immune system are responsible for coordinating the body's response to such pathogens or abnormalities. At first glance, it may seem surprising to learn that psychologists would be so interested in understanding the immunity and immune processes. However, as the last several decades of research has revealed, there are extensive and complex relationships between psychological phenomenon and immunological processes. In 1964, Solomon and Moos first coined the term psychoimmunology to describe the field that examines interactions between psychological states and immune function. A short while later, the term was expanded to psychoneuroimmunology (PNI) to reflect the role of the neuroendocrine system in also linking psychological states and immunological activity. The immune system is directly innervated by the ANS (lymph nodes, spleen, thymus) and immune cells have receptors for catecholamines and glucocorticoids.

TABLE 4.4 Example Social Psychological Research Applications Utilizing Hormonal Indices

Topic	*Hormone Parameter(s)*	*Citation*	*Description*
Aggression and dominance	Testosterone (and cortisol)	Sherman et al., 2016	Salivary testosterone predicted greater number of subordinates for male executives, but only for executives with low cortisol.
Memory	Epinephrine, cortisol	Cahill et al., 1994	Compared to placebo, propranolol (which blocks epinephrine effects), impaired memory for emotional but not neutral material.
Prosocial behavior	Oxytocin, vasopressin	Poulin et al., 2012	Oxytocin and vasopressin receptor genes interacted with perceived threat to predict prosocial behavior in a representative U.S. sample.
Resilience	Dehydroepiandrosterone	Petros et al., 2013	Self-reported resilience was positively correlated with salivary dehydroepiandrosterone.
Social-evaluative threat	Cortisol	Dickerson & Kemeny, 2004	This large meta-analysis suggested that social-evaluative threat elicits greater salivary cortisol responses than non-evaluative situations.

In addition, the immune system communicates directly with the central nervous system. Thus, the field of PNI has been shaped by the interest in understanding the bidirectional relationships between psychological phenomenon and immune processes and disease.

Humans have a range of complicated physiological mechanisms to protect our bodies against the invasion and potential damage from foreign microorganisms and own self cells that have gone awry. The overarching function of the human immune system is to protect the body from disease-causing microorganisms and other potentially harmful substances as well resist and eliminate infected and abnormal cells, such as tumors. The immune system recognizes the surface molecules, or antigens, of foreign and host cells. Carrying out these important functions are two major integrated subdivisions of the immune system: innate immunity and adaptive immunity (Daruna, 2012). Evolutionarily older, innate immunity is often described as the nonspecific first line of defense against injury

and infection. Innate immunity is a broad system of action that can respond relatively rapidly (i.e., minutes to hours) to microbes and toxins. Innate immune responses are relatively short in duration; thus, they do not lead to long-lasting immune protection. Innate immunity is comprised of elements with which a person is born, always present and available to protect an individual. Innate actions include the body's physical barriers (skin), secretions (mucous, stomach acid), and mechanical processes to block, trap, inactivate, or expel pathogens. The innate system can recognize foreign substances and recruit other immune cells and molecules to initiate, maintain, or dampen inflammation.

The primary signaling molecules of innate inflammatory responses are pro-inflammatory cytokines. Pro-inflammatory cytokines are produced by immune cells and other cells of the nervous system. Beyond influencing local inflammatory processes, cytokines are responsible for regulating complex and far-reaching changes throughout the body and brain (Maier & Watkins, 1998). The name for such a widespread response to infection is often referred to as sickness behavior or the acute-phase response, and encompasses a variety of physiological, cognitive, behavioral, and affective changes. Cardinal physiological alterations include fever, alterations in sleep structure, and production of acute-phase proteins and white blood cells. Behavioral responses include social withdrawal or isolation, increased pain complaints, and reductions in physical activity, eating, and libido. Dysphoria and anhedonia are also observed. Cognitive alterations include deficits in attention and memory interference. Sickness behaviors are thought to be adaptive changes that develop during the course of an infection to influence an organism's motivational state, to reorganize priorities to best cope with pathogens.

The second branch, adaptive immunity (sometimes referred to as specific or acquired immunity), comes online when innate immune processes are not sufficient to address the infection and contact is made by the invading antigen. Due to the large number of possible antigens, the adaptive immune system generally has relatively few immune cells (i.e., lymphocytes) that can respond to any particular antigen. However, once the immune cell binds to its target, additional cells proliferate to mount a sufficient response to the infection. Depending upon which type of molecule is detected, distinct immune response occur; for instance, cell-mediated responses primarily occur to neutralize viruses that have invaded the host's cells or to eliminate abnormal self cells (e.g., cancerous cells); in contrast, humoral or allergic responses largely target foreign extracellular substances, such as bacteria and allergens. The process of a full proliferative response can take much longer (weeks in some cases), but the response can lead to long-term protection by leaving behind memory cells that may allow for a more efficient response upon subsequent exposure. Such immunological memory helps to explain how vaccinations work. For greater detail on the complexity of immune processes and the many cells, signaling molecules, and receptors that are of relevance, see Daruna (2012).

Assessment

Just as the process of immunity is complex, so is the assessment of the system's varied cells and processes. There is no "one" test to measure global, or overall, immune function. Rather there are a number of tests from which to choose to estimate various components and functions of the immune system (Kiecolt-Glaser & Glaser, 1995). At the time of this writing, the mostly widely used approaches of measuring different aspects of immune function in psychological and behavior research involve the collection and assaying of circulating blood samples. Circulating blood samples can allow for both enumerative assays and functional assays. See Table 4.5 for a summary of functional and enumerative psychoneuroimmune measures. Enumerative assays refer to the counts, percentages, or concentrations of specific immunological marker (e.g., total or specific lymphocytes, particular antibodies, cytokines or acute-phase proteins) typically quantified from blood samples and in some cases saliva. For example, greater levels of circulating acute-proteins (e.g., C-reactive protein) are interpreted as greater levels of inflammation. In contrast to enumerative assays, functional assays allow researchers to quantify immunological responses to a variety of challenges *in vitro* or *in vivo*. For example, a researcher may be interested in measuring natural killer (NK) cell activity. NK cells are important for the detection and destruction of tumor cells and virally infected cells. To test NK cell lysis, or NK cells' ability to destroy, or lyse, cells, an *in vitro* functional immune test is used. Blood samples are drawn from the participant and then separated from his or her blood in the laboratory. NK cells are then cultured in media with a radioisotope. Afterward, the tagged NK cells are incubated with target cells that they can kill (typically from a cancer cell line), thus releasing the isotope, which is subsequently measured by the researchers. Greater counts reflect increased NK cell efficacy, which has implications for cancer progression. Although blood samples can be used to test many different enumerative and functional immune parameters, it is important to recognize that such samples reflect peripheral processes, rather than localized processes; that is, much of the action in an immune response is localized to tissues and immune organs (e.g., lymph nodes). As such, blood draws may miss important information.

For all aforementioned immune tests (and those described in Table 4.5), specially trained staff and specific equipment is necessary to complete the assessments. Since many psychologists do not themselves have access to a nurse or phlebotomist and wet laboratory environment, it is important to partner with those who do have these skills and resources. As an alternative to blood-based immune tests and the constraints that come along with them, salivary assays of immune parameters have grown more common among behavioral scientists. For example, a recent review of the extant literature indicates that some cytokines (e.g., interleukin-1b, tumor necrosis factor alpha, and interleukin-6), increase

TABLE 4.5 Examples of Functional and Enumerative Psychoneuroimmune (PNI) Measures

PNI parameter	*Description*
Enumerative Measures	
Inflammatory cytokines	Test to measure amount of inflammatory signaling molecules (e.g., interleukin-6, tumor necrosis factor alpha)
Acute-phase molecules	Test to measure amount of acute-phase molecules produced in response to inflammation (e.g., C-reactive protein)
Specific antibodies	Test to measure specific antibodies (e.g., secretory immunoglobulin A)
Specific immune cell count	Test to measure number of specific immune cells (e.g., CD4+ cells)
Total white blood cell count	Test to measure number of circulating white blood cells, or leukocytes
Functional Measures	
Proliferative responses to challenge	Test to determine the degree to which lymphocytes proliferate or replicate in response to mitogens, or infectious agents, such as Lipopolysaccharide (and measure resulting increase in inflammatory cytokines, such as interleukin-6) or Concanavalin A or Phytohemagglutinin (and measure T- and B-lymphocyte responses, respectively)
Natural killer cell activity	Test to determine the cytotoxicity, or tumor-killing ability of NK cells, by incubating participants' NK cells with target cells. Degree of NK activity is quantified by amount of isotope released by lysed (killed) cancer cells.
Latent virus titers	Test to determine the immune system's ability to control a latent viral infection, such as herpesvirus or Epstein-Barr virus.
Response to vaccine	Vaccine (e.g., trivalent influenza vaccine) is administered to participants. Pre- and post-vaccine measures (via blood or self-report) are taken.
Response to inoculation	Virus (e.g., common cold) or bacteria is administered to participants. Pre- and post-inoculation measures are taken.
Wound healing	Wound (e.g., mouth puncture, skin abrasion) is administered to participants. Pre- and post-wound measures are taken.

fairly consistently in saliva in response to acute stress (Slavish, Graham-Engeland, Smyth, & Engeland, 2015).

Regardless of whether the researcher is collecting blood or saliva samples for quantifying aspects of the immune system, there are a number of methodological issues that are of relevant across all PNI research. Segerstrom and Smith (2012) describe three kinds of important error in PNI research: the good, the bad, and the ugly. The good error reflects variability in immune parameters that are of

interest to researcher. For instance, immune cells or inflammatory responses may vary as a function of psychosocial stress, pain, or other social psychological phenomena. Bad error is that which results from type I error, such as conducting studies with small sample sizes and many dependent variables. With the relatively high costs of conducting PNI research, sample sizes are often underpowered to perform complex statistical tests. Ugly error results from noise in the research process. For example, not controlling for time of day, using multiple assays, laboratories, or technicians to process samples, and improperly storing samples can all lead to error in immune parameters.

Example Research Applications

As with measures of the ANS and endocrine system, psychological stress is a major area of emphasis in understanding psycho-immune links. For example, in a series of studies by Sheldon Cohen and colleagues, psychological stress predicts greater susceptibility to developing the common cold and degree of symptom severity (e.g., Cohen, Tyrrell, & Smith, 1991). In addition, the experience of acute psychological stressors is reliably linked to increased plasma and salivary markers of inflammation (Segerstrom & Miller, 2004; Slavish et al., 2015). Other lines of research indicate that pro-inflammatory cytokines regulate psychosocial behaviors, such as social withdraw, and other sickness behaviors (anhedonia, dysphoria, fatigue, and cognitive and motor impairment). For example, research by Naomi Eisenberger and colleagues reveals how inflammation contributes to social, cognitive, and affective symptoms of depression (e.g., Eisenberger, Inagaki, Mashal, & Irwin, 2010). See Table 4.6 for additional examples of social psychological research with immune measures, including studies of emotional disclosure, perseverative cognition, and personality.

General Methodologic and Analytic Considerations

If it has not yet been made abundantly clear from the preceding sections, psychophysiological assessment is not for the faint of heart! When one begins to undertake the acquisition of biological data, many questions need to be answered, starting with: Which system should be measured? Which biomarkers within a given system? Once the system or biomarker has been identified, next questions are: What kind of equipment, staff, and training are needed for the acquisition of these data? What timing and number of assessments should be recorded? Alongside these questions are other practical considerations, including cost, equipment, space, degree of invasiveness and burden placed on the participant, availability of appropriately trained collaborators and research assistants, and procedures for storing, analyzing, and interpreting the data. The following sections discuss several broad methodologic and analytic issues that arise when collecting psychobiological data.

TABLE 4.6 Example Social Psychological Research Applications Utilizing Psychoneuroimmune (PNI) Measures

Topic	*PNI Parameter(s)*	*Citation*	*Description*
Emotional disclosure	Wound healing	Weinman et al., 2008	Compared to a control condition, an emotional disclosure writing intervention led to smaller punch biopsy wounds.
Perseverative cognition	Acute-phase molecules, inflammatory cytokines	Zoccola et al., 2014	Compared to distraction, post-stressor rumination led to greater C-reactive protein (but no difference in interleukin-6 or tumor necrosis factor alpha).
Personality	Response to cold virus	Cohen et al., 2003	Positive emotional style was associated with lower risk of developing a cold after rhinovirus exposure.
Social disconnection	Inflammatory cytokines	Eisenberger et al., 2010	Relative to placebo, endotoxin led to increases in interleukin-6 and tumor necrosis factor alpha as well as greater feelings of social disconnection.
Stress	*many*	Segerstrom & Miller, 2004	The large meta-analysis indicates that stress reliably alters immune parameters, and the nature of the alterations depend on the nature of stress (e.g., acute versus chronic).

Tonic versus Phasic Measures

Although some hypotheses are concerned with a tonic physiological parameter (e.g., resting HR variability), others are address phasic responses (e.g., short term changes in cortisol concentration). Tonic levels refer to relatively stable values of a particular parameter. Tonic measures are sometimes referred to as resting or baseline levels (e.g., resting HR). Phasic measures refer to momentary fluctuations, typically occurring in response to some type of stimulus that has been introduced by the researcher. Phasic measures are sometimes also referred to as reactivity or responsivity (e.g., skin conductance response, HR reactivity). When testing the latter, it is vital to select appropriate comparison conditions (e.g., resting baseline) from which to derive a response. Sometimes a minimally demanding, or "vanilla," baseline period in which the participant is alert but inactive is a more appropriate comparison than absolute rest (Jennings, Kamarck, Stewart, Eddy, & Johnson, 1992). A true "resting" basal period can be quite difficult to achieve, and participants who are asked to do "nothing," may be anxiously awaiting the next

laboratory procedure, dozing off, or trying to find some other distracting activity to occupy them selves in the laboratory.

Timing and Number of Assessments

Biological changes in the autonomic, endocrine, and immune system vary in speed and duration. ANS changes can occur in fractions of a second, whereas it may take many minutes or hours to see full endocrine or immunological responses to particular stimuli. Additionally, psychological phenomena vary in their temporal characteristics and dynamic complexity (e.g., fleeting or fluctuating emotions, chronic ongoing stress). Researchers should carefully consider the temporal characteristics of the phenomena under study to ensure that the timing of assessment of psychological and biological measures are appropriately aligned. It bears noting, however, that with new measures and questions, there can often still be a bit of guesswork in selecting psychobiological sampling periods. Recent and future empirical and meta-analytic work will be useful in helping to establish such windows of time. For example, how soon after the introduction of a psychosocial stressor might a researcher expect to observe peak changes in salivary cortisol? Results from a meta-analysis of 204 laboratory stressor tells us that we should aim for 21–30 minutes post-stressor onset (Dickerson & Kemeny, 2004).

Given that some psychobiological data are collected continuously over extended periods of time, such as over the course of an hour-long laboratory visit with multiple tasks or over several days in an ambulatory study, researchers need to make decisions about how to quantify continuously collected data. What periods of time are of interest? The last minute of a resting baseline period? Each minute of a 5-minute cognitive task? The average of an entire task period? Answers to these questions should be dictated by the research questions of interest (and limits of psychophysiological recording equipment!). If the researcher plans to calculate difference scores to quantify change, or compare ANS activity during one procedure versus another, he or she should select sampling periods, or epochs, of equal duration so that variance estimates are comparable.

Data Storage, Processing, Analysis, and Interpretation

Data Synchronization and Storage

The synchronization of data across measurements for each individual is critical for analysis and interpretation. Careful timing and marking of continuous and intermittent data files is essential to compiling full datasets that link task or stimulus outcomes to physiological data. In addition to consulting with hardware and software companies that supply the products used in the laboratory, it may be necessary to consult with additional experts with backgrounds in biotechnology or software programming to facilitate this process. Furthermore, it is good practice

to pilot test data collection procedures and then practice data extraction, compilation, and analysis to ensure all necessary variables are properly collected and stored. Pilot testing is also necessary to ensure sufficient time for participants to habituate to the laboratory, complete tasks, and capture peak reactivity or recovery data.

Data Ranges and Norms

Many physiological parameters fluctuate over time as result of multiple factors, including variables of theoretical interest (e.g., emotional state) or as a result of methodological issues (e.g., movement, temperature, time of day). Once psychobiological data are collected, it should be inspected for possible artifacts and biologically implausible values (e.g., increased HR due to finger tapping). Although analysis software typically has algorithm-based functions to identify and address artifacts in continuous waveform data, it is useful to visually inspect data to some extent. Thus, it is important to train conscientious research assistants to assist in this biosignal data processing and editing role. In some cases, meaningful clinical cut-offs can be used when processing psychobiological data (e.g., systolic blood pressure over 140 may signify hypertension). Often, psychobiological data are non-normally distributed; positive skewness and outliers are common. For some, outliers may be of interest and relevant to the research question, so excluding extreme values may not always be the right approach. Nonetheless, data transformations are common (e.g., log transformations of salivary cortisol), but can make it more difficult to interpret findings. It is generally a good idea to become familiar with reporting guidelines and norms for your psychobiological measure of interest.

Statistical Analysis

Psychobiological research typically involves repeated measures or continuous assessments that are subsequently broken down into time-series data for each participant. Such data violate key assumptions of independence of many statistical methods. Despite this, statistical techniques based on analysis of variance (e.g., repeated measures ANOVA) with appropriate corrections for assumption violations are still the most commonly used in psychobiological research at present. Increasingly more common are empirical papers that contain regression-based techniques and multi-leveling modeling analytic procedures. For example, Houtveen, Hamaker, and Van Doornen (2010) detail a multilevel path analysis approach for analyzing 24-h ambulatory physiological recordings. Researchers interested in psychobiological research should consider taking advanced statistical courses at their universities or attend such workshops elsewhere. In addition, there are a variety of helpful resources analyzing psychobiological data with multilevel models (e.g., Blackwell, Mendes de Leon, & Miller, 2006; Hruschka, Kohrt, & Worthman, 2005).

Interpretation of Results

What does it mean if a biological parameter is moderately correlated with psychological variable? And what if they don't correlate? To some extent, the answer to this question will depend on the particular theory driving the research. However, alternative biological and methodological explanations are also worth considering and addressing in future research. For example, a review of 49 studies employing a standardized psychosocial laboratory stressor found small to moderate associations between biological and emotional stress responses in approximately 25% of the studies (Campbell & Ehlert, 2012). Because synchrony of physiological and psychological measures in response to stress was expected based on theory, the authors concluded that a variety of methodological, psychological, and biological factors may have contributed to the weak convergence in measures (e.g., socially desirable responding, poorly timed assessments, novelty of task). One recommendation stemming from this review is that rather than relying on single post-task or pre-post assessments, researchers might be wise to implement and aggregate across multiple assessments (e.g., across multiple tasks; in response to repeated same task exposure) to reveal trait-like physiological response dispositions.

Concluding Remarks and Future Directions

To date, the majority of research incorporating psychobiological measurement has taken place in research laboratories. Although this approach has led to a variety of important insights, idiographic longitudinal studies are best suited to track how alterations in one system or set of processes may covary with changes in another. For example, daily diary designs with ambulatory biological assessment are ideal to study the dynamic unfolding of stress and coping processes (e.g., stress appraisals, emotions, and concomitant biological changes). Moreover, ambulatory physiological assessments in naturally occurring contexts can allow researchers to more fully understand the experience of people by capturing life as it is lived. Future research that incorporates both laboratory-based and ambulatory assessments could lead to transformational research findings and help address limitations that exist in laboratory-only or field-only designs. Fortunately, a variety of technological advances in high-capacity batteries, compact design and portability, miniaturized sensors, powerful computing, and easy-to-use software will allow for long-term continuous measurement in daily life with minimal disruption. Substantial efforts also are being made to produce biological sensors that are smaller and allow for more continuous wearable sensors that can reliably quantify biomarkers in perspiration (Jia, Chew, Feinstein, Skeath, & Sternberg, 2016). Still other new technologies allow for sensors to be swallowed, printed on skin, or imbedded in already worn accessories (e.g., eye-glasses, jewelry). For a review of a variety of wearable sensors, see the Department of Defenses' recent report (Hirschberg, Betts, Emanuel, & Caples, 2014).

As technology continues to advance, so too will the opportunities for innovative psychobiological assessment by social scientists. The marriage of sound psychological theory with deep understanding of physiological systems are necessary for the most insightful and impactful research. Successful scholars of psychobiological assessment will be those who are both well-versed in social psychological theory and have a good understanding for human physiology (both its function and assessment). That said, it is readily acknowledged that any one person will not have full knowledge of all biological systems and processes that may relevant to the research question at hand. Thus, collaborative science is truly necessary in this realm of research. Is psychobiological assessment worth the trouble? The answer is a resounding yes. Although psychobiological assessment may not be suited for all social psychological research questions or researchers, it has the ability to complement and enhance existing social psychological methods, and lead to new insights to understanding human mind and behavior.

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