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New Approaches: Non-Invasive Cardiac Psychophysiology as a Tool for Translational Science with Marmosets

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Abstract

The importance of marmosets for comparative and translational science has grown in recent years because of their relatively rapid development, birth cohorts of twins, family social structure, and genetic tractability. Despite this, they remain understudied in investigations of affective processes. In this methodological note, we establish the validity of using non-invasive commercially available equipment to record cardiac physiology and compute indices of autonomic nervous system activity – a major component of affective processes. Specifically, we recorded electrocardiogram and impedance cardiogram, from which we derived heart rate, respiration rate, measures of high frequency heart rate variability (indices of parasympathetic autonomic nervous system activity) and ventricular contractility (an index of sympathetic autonomic nervous system activity). Our methods produced physiologically plausible data, and further, animals with increased heart rates during testing were also more reactive to isolation from their social partner and presentation of novel objects, though no relationship was observed between reactivity and specific indices of parasympathetic or sympathetic nervous system activity.

Keywords

affect; autonomic nervous system; respiratory sinus arrhythmia; heart rate variability; ventricular contractility

Introduction

One of the major challenges facing translational and comparative science is the identification of tools that can be deployed in multiple species without anthropomorphism. This is a particular challenge for affective science because we are unable to ask nonhuman animals to verbally report how they feel and self-reports are still the gold standard for understanding

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humans' emotions. There is a long history in human affective science of using autonomic physiological measures as indices of affective and emotional processes (for recent reviews see, Kreibig, 2010; Mauss & Robinson, 2009; Siegel et al., 2018). Activity in the autonomic nervous system is thought to be a critical component of affect -- an omnipresent neurophysiological state that is characterized by some degree of pleasantness/unpleasantness and some degree of arousal/activity -- which forms the basis of emotions like happiness, sadness, anger, and fear (Barrett & Bliss-Moreau, 2009; Critchley & Nagai, 2012; Russell, 2003).

Luckily, many autonomic physiological measures that are used in humans can also be deployed in nonhuman animals because of the conservation of the autonomic nervous system across phylogeny, especially within primates (Kawashima, Sato, Akita, & Sasaki, 2005; Kawashima, Thorington, & Whatton, 2009). While there is some history of recording these signals in nonhuman animals, rarely do scientists differentiate activity of the two major branches of the autonomic nervous system (ANS) (e.g., measures of heart rate reflect activity in both branches; Bernston, Quigley, & Lozano, 2007) (for a review, Bliss-Moreau et al., in prep). Methods exist to non-invasively record indices that map specifically to parasympathetic or sympathetic nervous systems (PNS and SNS, respectively) activity that can be carried out easily in nonhuman primates by measuring the function of the heart, although these measures have never been utilized in the common marmoset (*Callithrix jacchus*). The goal of the present work was to establish methods to record data allowing for the computation of these indices in common marmoset monkeys so that they can be deployed in future work as translational tools to speed discovery in affective science. By expanding the species used in affective science, we can begin to unpack how and why affective processes evolved and also open the opportunity for better models of human and animal health processes.

Nonhuman primate models of human health and disease are critical for the development of effective interventions and treatments to improve wellbeing. Unlike experimentation in humans, nonhuman primate models provide unique experimental control, the ability to manipulate the system and environment – resulting in capacity for casual modeling – all while maintaining a remarkably close genetic relationship with humans (Capitanio & Emborg, 2008; Phillips et al., 2014; Ringach, 2011). Marmosets have long been an important model for a variety of fields including the study of social behavior and emotion, aging, obesity, toxicology, stem cell research, and neurological diseases, among many others (for reviews, see Okano, Hikishima, Iriki, & Sasaki, 2012; Ross, Davis, Dobek, & Tardif, 2012; Smith, Trennery, Farningham, & Klapwijk, 2001; Snowdon, 2001; Tardif et al., 2009). Marmosets can now be genetically manipulated via transgenic techniques (Sasaki et al., 2009) allowing for the possibility of conducting state of the art research targeting particular genetic mutations and ultimately developing interventions and treatments to treat those diseases. Given these recent advantages, many scientists foresee the popularity of the model increasing in the future (Okano et al., 2012; Servick, 2018). In fact, as of 2003, marmosets were the most commonly used nonhuman primate in research in Europe (Abbott, Barnett, Colman, Yamamoto, & Schultz-Darken, 2003) and the number of U.S. marmoset research colonies had more than tripled since 2009 (Servick, 2018). Separate from the translational relevance of work with nonhuman primates to human health issues, comparative research

with different species of nonhuman primates sheds light on how and why behavioral and biological phenomena evolved.

Compared to the rhesus macaque (*Macaca mulatta*), arguably the most widely used nonhuman primate in research worldwide (Carlsson, Schapiro, Farah, & Hau, 2004), marmosets offer a number of significant advantages for comparative and translational affective science. Marmosets thrive in a laboratory context because they are easily maintained in small family groups reflective of their natural group composition: a monogamous male-female pair and offspring (Abbott et al., 2003). While the small group size makes them easier to manage in a laboratory setting than macaques, it is also may be more consistent with human family social structure, allowing for potential comparative study of the impact of family interactions on affective processes. Additionally, marmoset births often produce twins or triplets (Abbott et al., 2003). This allows for the study of variation in developmental trajectory from animals who shared the same fetal and developmental environment. Marmosets develop at a much faster rate than rhesus monkeys, allowing for womb-to-tomb study of affective processes over a short period of time. Despite these benefits, compared to other study species, there have only been a small number of experimental studies investigating affect or emotional processes in marmosets and none of these have included non-invasive cardiac physiological recordings as dependent variables (for a review see Oikonomidis et al., 2017). For example, a number of studies have used urinary or fecal hormone assays to evaluate affect-related processes including response to early social stress (Dettling, Feldon, & Pryce, 2002a; Dettling, Feldon, & Pryce, 2002b; Pryce, Dettling, Spengler, Schnell, & Feldon, 2004), aggressive responding to social intruders (Ross & French, 2011), social isolation (Smith & French, 1997; Galvão-Coelho, de Menezes Galvão, da Silva, de Sousa, 2017) and manual restraint (Smith & French, 1997). Studies using implanted cardiac physiological recordings have investigated responses to familiar and unfamiliar social partners (Gerber, Schnell, & Anzenberger, 2002), anticipation and rewards (Braesicke et al., 2005), and the neurobiology of affective learning (Agustín-Pavón et al., 2012; Wallis, Cardinal, Alexander, Roberts, & Clarke, 2017).

Some evidence does exist indicating that cardiac function in marmosets can be recorded using non-invasive tools. Work from Ghazanfar and colleagues (Borjon, Takahashi, Cervantes, & Ghazanfar, 2016; Liao, Zhang, Cai, & Ghazanfar, 2018) investigating the relationship between ‘arousal’ and vocalizations recorded electromyography (muscle movement) related to vocalizations and computed an index of heart rate from that. They document that the measure is sensitive to variation in heart rate across social conditions and related to the generation of vocalizations, but the measure does not provide the same fine-grained information about heart cycle (including the points that are required to compute sympathetic indices such as pre-ejection period). In order to evaluate vocalizations relative to respiratory cycle to determine if some variation is due to respiratory sinus arrhythmia, Borjon et al. (2016) built statistical models to evaluate the interplay between heart rate and respiration cycle – but did not calculate an index of respiratory sinus arrhythmia per se. Their success at recording the electromyography signal with surface electrodes in awake marmosets supports the *a priori* plausibility of our methods.

In order to further develop the marmoset as a model species for affective science, the present study evaluated the feasibility of non-invasively indexing cardiac activity in the autonomic nervous system. Nonhuman primates are a good model for cardiac evolution due to the highly conserved nature of the system across phylogeny (Kawashima et al., 2009). Heart anatomy and cardiac innervation is relatively consistent across species within the primate order, meaning that the ANS regulates the cardiac system in much the same way in nonhuman primates and humans. The classic belief, broadly documented in biology and psychology textbooks, is that the PNS controls the “rest and digest” functions of the body, while the SNS controls “fight or flight” responses. Modern evidence supports an alternative hypothesis - the two branches of the ANS work together to co-regulate homeostasis (Berntson, Cacioppo, & Quigley, 1991). What that means is that measurements of the PNS and SNS provide unique information about the function of the organism (unlike heart rate, which reflects a blend of PNS and SNS activity). Our laboratory has previously demonstrated that ANS indices, specifically respiratory sinus arrhythmia (an index of PNS activity) and pre-ejection period (an index of SNS activity), track with the valence (hedonics) of affective experience in rhesus macaques opening up the possibility of using ANS measures as indices of affective processes in nonhuman primates (Bliss-Moreau, Machado, & Amaral, 2013)

Indices of PNS and SNS activity can be computed via the analysis of electrocardiogram (ECG) and impedance cardiogram (ICG) data which can be easily recorded using non-invasive surface electrodes designed for recording the signals in humans. High frequency heart rate variability is the variation in inter-beat intervals (IBI; time between R-spikes on an ECG) during inhalation and exhalation is mediated by vagal pathways and is a reliable measure of PNS activity (Berntson et al., 1997; Brownley, Hurwitz, & Schneiderman, 2000; Porges, 2007). It is typically evaluated via two different calculations, both derived from ECGs. The first calculation of high frequency heart rate variability is via spectral analyses which decompose variation into its frequency components and then evaluate variation in the frequency range consistent with respiratory frequency (i.e., respiratory sinus arrhythmia, RSA) (Berntson et al., 1997). Additionally, variation in the timing between beats can be computed as the root-mean square of the successive differences of the IBI series (RMSSD) – a measure which does not account for the respiratory cycles specifically but has been previously used in marmosets (Wallis et al., 2017). Both RSA and RMSSD are measures of high frequency heart rate variability, thought to reflect PNS activity, and are expected to be highly correlated (Allen, Chambers, & Towers, 2006; Berntson et al., 1997; Berntson, Quigley, Norman, & Lozano, 2016; Camm et al., 1996), although RMSSD may be less influenced by respiration (Shaffer & Ginsberg, 2017). Pre-ejection period (PEP), the time between the heart being signaled to beat and aortic opening, is an index of ventricular contractility which reflects SNS activity (Berntson et al., 2016). It is computed from the cardiac impedance signal which measures the impedance of a weak electrical signal passed between two electrodes placed on the chest between the clavicle notch and xyphoid process – because the aorta is the primary large vessel within that area, impedance across the space reflects blood flow in the aorta. Low frequency variation in the impedance signal, once thought to be “noise”, reflects respiration and thus respiration rates and cycles can be derived from the impedance signal as well (Ernst, Litvack, Lozano, Cacioppo, & Bernston,

1999). Of note, ECG and ICG data can be used to compute a number of other metrics that reflect various aspects of autonomic function not discussed here (Berntson et al., 2016).

Given that we have previously established methods for non-invasively collecting ECG and ICG data and computing indices of high frequency heart rate variability (namely RSA) and PEP in rhesus monkeys (Bliss-Moreau et al., 2013). Our objective was to add to the growing toolbox of methodologies for comparative and translational affective science, specifically non-invasive measurements of cardiac physiology in nonhuman primates. Here we show that RSA and PEP can be feasibly measured in the common marmoset using non-invasive tools (i.e., surface electrodes rather than implanted telemetry) and render values that are physiologically plausible.

Description

Noninvasive cardiac physiological recordings were carried out using standard Ag-AgCl surface electrodes attached to a MindWare mobile unit (MindWare Technologies LTD. Model #: 50-2303-01) to record both electrocardiogram and impedance cardiogram data. As a proof of concept, we evaluated cardiac data and behavioral data in a group of socially-housed adult marmosets at the Barshop Institute for Longevity and Aging Studies, University of Texas Health Science Center at San Antonio. All procedures were approved by the University of Texas Health Science Center, San Antonio Institutional Animal Care and Use Committee. This research adhered to the American Society of Primatologists Principles for the Ethical Treatment of Non-Human Primates and the *The Guide* (National Research Council (US) Committee for the Update of the Guide for the Care and Use of Animals, 2011).

Methods

Subjects.—Subjects were 15 adult male common marmosets (*Callithrix jacchus*), ranging in age from 1.6 to 15.3 years, weighing 442 ± 13.31 grams (mean ± SEM). All animals were socially housed in pairs or family groups (Layne & Power, 2003) and tested individually as described below. Physiological data collection occurred prior to behavioral data collection for all animals. All testing occurred over a three-day period.

Physiological Data Collection and Processing.—Animals were transferred from their home cages to the testing room in standardized transport boxes. In the testing room, were removed from the transfer box by an experienced, familiar, experimenter (CR) wearing leather capture gloves. Their chests and two spots on their back were shaved using clippers with 40 gauge blades. Shaved areas were cleaned with 70% ethanol on gauze and allowed to air dry. Data were collected at 1000 Hz, using a MindWare Mobile unit (MindWare Technologies LTD. Model #: 50-2303-01) and disposable Ag-AgCl spot electrodes (Huggable Pediatric Electrodes; ConMed Inc).

ECG electrodes were placed in a standard modified Lead 2 configuration (one electrode on right shoulder, one electrode on the lower left rib, and the ground on the lower right rib) and IGC electrodes were placed in the standard configuration (ventral surface at base of neck at the clavicular notch and on the xiphoid process; dorsal surface ~ 2.5 cm above the ventral

top electrode and ~ 2.5 cm below ventral bottom electrode). After many attempts at attaching electrodes, we determined that the most efficient process was to attach electrodes to their ventrums and then their backs. The experimenter wearing gloves (Experimenter 1) gently restrained the animals' arms and legs, positioning them on their backs on an exam table, while a second experimenter (Experimenter 2) attached the front electrodes. Once the electrodes were attached, Experimenter 2 placed a piece of medical adhesive tape over each electrode. Note that when testing rhesus macaques, our team wraps their torsos in either a sticky disposable bandage or reusable velcro adhesive bandages to hold the electrodes in place (Bliss-Moreau et al., 2013) but this was not deemed necessary for marmosets. The animal was then placed chest down on Experimenter 1's hand so that the electrodes on the chest could be held in place and the marmoset rested in a quadrupedal position across the gloved hand. Experimenter 1 restrained the animal's head allowing Experimenter 2 to attach the back ICG electrodes. Once the electrodes were placed Experimenter 1 could restrain the animal in this position and stabilize the electrodes, which resulted in very few movement artifacts in the data. Initially, this process took a total of approximately five minutes to prepare the animals for data recording; although, with practice and experience preparation time decreased to ~2 min.

Data recording began when the electrodes were all placed and electrodes were adjusted as necessary (to ensure signal quality). Cardiac data were recorded for a minimum of three minutes. After data recording, electrodes were removed and animals were returned to the transport box and then their home cages.

Data were scored offline using MindWare tools (methods described in detail in Bliss-Moreau et al., 2013). Briefly, ECG data were scored using MindWare HRV (version 3.1.0), using the ICG signal (Zo) as a respiration signal. The ECG was visually inspected to ensure proper placement of R-spikes and to determine if segments needed to be discarded due to motion artifacts. We adopted a conservative approach and did not include segments with motion (or other) artifacts rather than editing the data; as a result, not all animals had three full minutes of cardiac data.

Analyses of cardiac data of these types require that the experimenter determine how the data is segmented for scoring as data are typically summarized across small periods of time. This choice is typically made based on some combination of the task design (e.g., if an individual is shown 30-sec movies, then the analysis window might be 30-sec to correspond to the movie length, as in Bliss-Moreau et al., 2013) and physiological properties of the species including how many heart cycles they generate on average per minute. Methodological guidelines developed for humans suggest that the time window minimum should be 10 times the length of the lowest frequency cycle (Berntson et al., 1997). Therefore, with respiration rates of on average 32.66 (or 1 breath every 0.544 seconds), the minimum segment, according to the literature, should be 5.44 seconds.

Initially, we scored all data in 15 second bins (or 'ensembles') meaning that each HR, RSA, RMSSD or PEP data point was based on 15 seconds of ECG and/or ICG data. Across all animals there were 203 possible 15-second segments and 13 of those segments were discarded (6.4%) because of motion or other artifacts.

We set the high frequency heart rate variability (i.e., respiratory sinus arrhythmia) band at 0.040 to 1.240 Hz based on physiologically established respiration rates in marmosets (Horii et al., 2002; Ludlage & Mansfield, 2003) and visual inspection of the respiratory signal. After visual inspection and correction of R-spikes (when necessary), data were read out from MindWare HRV to Excel files using the MindWare interface. This generates a record of data with a number of cardiac measures including heart rate, inter-beat interval, respiratory sinus arrhythmia, and other measures of heart rate variability such as RMSSD. Means for each index were computed (across 12 bins). Values that were greater than or less than 2 standard deviations from each animal's mean were dropped. Data were then aggregated to compute means and medians. Data were then rescored in 30 s and 60 s bins in order to compute the stability of the estimates at each bin length.

For a small subset of animal (N=5), we computed pre-ejection period (PEP) – the time between the Q-point in the ECG signal (depolarization of the heart septum, the onset of the heart beat) and the B-point (opening of aortic valve) in first derivative of the impedance signal (dZ/dt) (Berntson, Lozano, Chen, & Cacioppo, 2004; Lozano et al., 2007). MindWare hardware and software records both the impedance between placed electrodes (an index of impedance in the aorta) as well as the first derivative of that signal (dZ/dt) on which the B-point can be identified. Note that in some other hardware/software combinations only the Zo signal is recorded and dZ/dt must be mathematically derived from it. Our small sample subset resulted from data loss for the other animals which occurred because of poor quality higher frequency information in the dZ/dt signal as we learned to optimize electrode placement (note that the Zo signal was visually inspected and deemed acceptable for using as a respiration signal in the RSA analyses detailed above), motion artifacts, or a combination of the two. PEP data were scored offline using MindWare IMP version 3.1.6 (methods described in detail in Bliss-Moreau et al., 2013) in 15 second bins. Both Q and B points were manually placed as automated scoring procedures are not consistently accurate. As with the ECG scoring we adopted a conservative approach, excluding segments with motion (or other) artifacts.

Behavioral Data Collection and Processing.—On the same day as their physiological data collection all animals were tested in a novel object task. Adult male-female social pairs were moved to a quiet test room in their standard cages and separated such that the male test animal was in the top portion of the cage and the female animal was in the bottom portion of the cage. As such, they were in auditory and olfactory, but not visual contact for the duration the experiment. Cages included both a perch branch and nesting box.

Animals were tested in two conditions. Condition 1 always occurred first. In Condition 1, the animal was left alone in the test cage with no manipulation for 10 minutes. This condition was intended as a habituation period to the solo caging; review of the video tapes, however, suggested that the social isolation was a potent affect induction itself as animals' behavior was consistent across Condition 1 and 2. In Condition 2, a familiar experimenter (CR) placed a series of novel objects (never before encountered by these individuals) into the animals' test cage for 10 minutes. These objects were colorful or textured dog chew toys, one suspended from the cage ceiling via a clip and two placed on the floor of the cage.

During both Conditions 1 and 2, animals' behaviors were video recorded allowing for behavioral scoring offline.

Behavioral scoring was completed by one rater (CR) at a separate time using Noldus Observer Software, Version XT v14. Behavior was scored using a standardized ethogram for marmoset behavior (see Table 1; Ross, French, & Patera, 2004; Ross et al., 2012) that included position within the cage and exploratory, communicative, and anxiety related behaviors. Position in the cage was scored in 20 s intervals and exploratory, communicative, and anxiety-related behaviors were scored all occurrences. As in previous reports (Bliss-Moreau, Bauman, & Amaral, 2011; Bliss-Moreau & Moadab, 2016), we computed an "affective reactivity" index equal to the sum of all behaviors related to affective states and communication.

Data analysis.—Data analysis was performed in SPSS Version 25 (IBM Corp). We elected to use non-parametric correlations (Spearman) because of the small sample size and non-normality of some of the data. Visual inspection of the behavioral data indicated that animals were behaviorally reactive during both Condition 1 (empty cage) and Condition 2 (novel object). Therefore, we computed a global reactivity variable that reflected total affective reactivity during the entire behavioral test session.

Results

Heart Rate and Heart Rate Variability.—The non-invasive ECG recordings that we carried out produced physiologically plausible data, similar to reports of ECG data collected in anesthetized marmosets or those implanted with physiological recording devices (Davies, 1969; Gerber et al., 2002, Schnell & Wood, 1993; Wallis et al., 2017). In addition, the values for the heart rate variability measures – respiratory sinus arrhythmia (RSA) and root mean squared successive differences in the inter-beat interval series (RMSSD) – and respiration rate were also physiologically plausible, relative to heart rate. This evaluation was made based on studies of human children (whom have higher heart rates than human adults) and developmental changes in RSA and RMSSD as related to heart rate and IBI (Quigley & Stifter, 2006; Massin & von Burnuth 1997; Richards, 1994). See Table 2 for descriptive statistics.

Average RSA values were low and the range of RSA values were extremely restricted, most likely due to the high heart rates of the animals. Because RSA is an index of the variation in timing between R-spikes of the ECG (IBI timing), faster heart rates lead to reduced IBI durations which inherently restrict the magnitude of the variance, simply because the numeric values are low. That said, RSA and RMSSD were correlated as expected, r_s=0.895, p<0.0001 (Figure 1). Heart rate and IBI behaved identically in their relationships with the other physiological variables (correlation coefficients the same, with different signs) as expected given that they are computed from the same signal and both represent information about the timing of R-spikes. Heart rate was positively correlated and IBI was negatively correlated with respiration rate, r_s=0.532, p=0.041. Neither heart rate nor IBI were correlated with either RSA or RMSSD, r_s=0.407, p=0.132 and r_s=0.268, p=0.334, which is not unusual given that heart beats are co-regulated by both the parasympathetic and sympathetic nervous

systems (Bernston et al., 2007). Respiration rate was not correlated with either RSA or RMSSD, r_S=0.040, p=0.887 and r_S=−0.136, p=0.630.

Affective reactivity was not related to heart rate variability (either RSA or RMSSD) or respiration rate. That said, there was a positive correlation between heart rate and affective reactivity that did not reach conventional levels of significance, r_S=0.491, p=0.063 (see Figure 2), in all likelihood because of the small sample size. Affective reactivity was not related to movement around the cage, r_S=0.155, p=0.581, or the number of interactions the animals had with the objects, r_S=0.239, p=0.390.

There were no significant relationships between movement and the physiological variables (all ps> 0.49), but there was a significant relationship between movement and animals' interactions with the objects such that animals that moved more also interacted with the objects more frequently, r_S=0.643, p=0.010.

What Duration of Data is Required?: Typically, in psychophysiology, bin or ensemble duration (the time period over which the signal is counted or averaged to generate a single data point) is determined based on the number of physiological cycles in a given time period such that a scored epoch includes at least 10 cycles (Berntson et al., 1997). Continuous data streams are typically parsed into smaller time bins to compute point estimates, and then those time bins may be either used as the data points (e.g., when showing an animal a 30 s video, a 30-s scoring bin might be used; as in Bliss-Moreau et al., 2013) or aggregated across longer durations (e.g., to represent physiology during a 5 or 10 min test period; as in Bliss-Moreau, Moadab, & Capitanio, 2017).

Given the heart rate of the marmosets (on average 364.57 beats per minute), we had every expectation that 15 s bins would provide stable estimates of the cardiac variables. The advantage of using smaller bin durations is that fewer are lost to motion artifacts. To test this experimentally, we carried out a series of analyses in which data were rescored into 30 s bins and 60 s bins. The original 15 s bins were aggregated into 30 s averages and 60 s averages; the 30 s bins were averaged into 60 s averages. We then correlated the first minute of data with the second, the second minute of data with the third, and the first minute of data with the third and computed the intraclass correlation (ICC) of all 3 aggregates for both the 15 s and 30 s bins (Table 3a) and each 30 s aggregate of 15 s bins with 30 s bins (Table 3b) to assess the stability of the measures. Variation from 1 in the correlation coefficients therefore reflects variation in stability of the estimates across different time windows or data loss (i.e., variation due to reduced number of time bins included in the estimates when time bins are dropped because of artifacts). That is, if Minute 1 has a motion artifact between seconds 16 and 22, when scored as 15 s bins its average would include 3 bins because the bin including data between 16 and 30 seconds would be dropped, but when scored as 30 s bins would include only the value of the 2nd bin of data, because the first 30 s bin would be dropped due to the motion artifact.

We found that 15 s bins, aggregated into 1 minute provide a more stable estimate of the cardiac variables because the correlations are very high, while losing less data (3.89%) compared to 30 s bins (6.67%). That said, the correlations for the 30 s bins that were

aggregated into 1 m and their corresponding ICCs were high, especially for RMSSD and HR, suggesting that the best approach for a given analysis is that which is consistent with the study's goals and a priori hypotheses about the temporal nature of the studied phenomena. One possibility is that the computations of RSA in this software package are particularly impacted by the duration of the time bin.

Pre-Ejection Period.—Data from the five animals from whom we collected high fidelity dZ/dt data revealed physiologically plausible PEP times given the animals heart rates. The mean PEP across animals was 21.32 ms (*SD*=11.02) with a range of 9.17 to 28.11 seconds.

Comparison and Critique

The present report documents that it is possible to collect high fidelity cardiac data from marmosets using commercially available products that scale across species, non-invasively, while they are temporarily restrained. Measures of heart rate, heart rate variability, and pre-ejection period were all within physiologically plausible ranges. Electrocardiogram data were all of high fidelity for all animals and modification of the electrode placement and restraint protocol resulted in perfecting collection of impedance cardiogram data as well. While only male animals were tested in the current experiment, previous reports show no sex differences in marmoset cardiac physiology recorded via implanted devices when males and females were tested in various social and environmental conditions (alone or with pairmate in familiar or unfamiliar environment) (Gerber et al., 2002). Of note, the procedure used – physically holding the animals without a prior habituation phase – may have resulted in elevated heart rates, near the typical ceiling values for marmosets. The mean HR for our animals was 364.57, while Schnell & Wood (1993) reported diurnal rhythms ranging from 153 ± 4 to 259± 6 in animals freely moving in their home cage.

While a common dependent variable in affective science, heart rate is regulated by both the PNS and SNS. An increase in heart rate may mean an activation of the SNS, a deactivation of the PNS, or both (Berntson et al., 1991). Heart rate may, therefore, be a reasonable dependent variable for experiments that seek to document only that a change has occurred in the cardiac system as a result of, for example, perceiving a novel stimulus. Tools that allow for teasing apart which branch of the ANS is impacted may allow for more precise understanding of biological mechanism of psychological phenomena. The robustness of the ECG signal – we had nearly perfect signals in all of our subjects upon immediate placement of the electrodes, despite challenges recording ICG – allows it to be easily deployed in a variety of settings and species. As a result, we anticipate RMSSD could be easily used as an index of high frequency heart rate variability moving forward as it requires only information derived from the ECG. Further, future studies might evaluate specific features of the ECG wave form (e.g., Q-T timing).

The limited sample size and single behavioral assay prevent us from drawing strong conclusions about the relationship between behavior and physiology. While there were not statistically significant relationships between the PNS outcome measures and behavioral affective reactivity, there was a relationship between HR and behavioral reactivity such that, in general, animals with higher heart rates were also more reactive. The correlation

coefficient did not reach the p<0.05 cut off (at $p = 0.063$) but the correlation coefficient suggested a moderate relationship (0.49) and visual inspection of the data (see Figure 2) supported the conclusion that there was a relationship. It is possible that we would have observed a statistically significant result with a larger sample size. It is also possible that we would have observed more pronounced individual differences in animals that were habituated to the procedure or tested without being held and thus HR was not near ceiling. That said, the finding that HR is elevated in animals who are more behaviorally reactive is consistent with the nonhuman primate psychophysiology literature more generally (Bliss-Moreau et al., in prep, for a review) and findings from marmosets specifically. For example, marmosets who are unable to learn to discriminate between two stimuli – one with affective significance and one neutral stimulus – in a classical conditioning task were more behaviorally affectively reactive, as indicated by reduced locomotion and increased head-cock, and had higher heart rates when confronted with a rubber snake (Shiba et al., 2014).

Establishing the use of non-invasive cardiac psychophysiological measures as translational tools for the study of affective processes in nonhuman primates, generally, and marmosets, specifically, is an important step for furthering translational affective science. The need for improved translational models of human affective health and pathology is clear; additionally, improved techniques for monitoring nonhuman animal affective health and pathology are necessary. Mood related psychopathology remains a significant concern in human populations, as does caring for the welfare of nonhuman animals in our charge, both in research and zoo environments. Marmosets are an increasingly popular model for human behavior and physiology (Abbott et al., 2003; Okano et al., 2012). Psychological researchers in particular have an untapped resource in the common marmoset as they age faster than both human and rhesus monkey subjects, live in social groups more similar to human families than other popular animal models, and are almost always born as twins, allowing for studies of genetic impacts on behavior (Abbott et al., 2003; Haig, 1999). Having a robust translational tool kit that allows for experimentation across species, using the same tools without adaptation or anthropomorphism, is vital to understanding the evolution of psychological processes (Bliss-Moreau, 2017). Due to its highly conserved nature, cardiac measures of affect such as high frequency heart rate variability and PEP are some of those tools. Using these methods it is possible to collect these data non-invasively, reducing the logistic and financial burden on researchers compared to implanted telemetry. In this vein, deploying the methodology discussed in this paper also speaks to Russel & Burch's (1959) Three R's of animal research ethics insofar as it eliminates the need for animals to undergo surgery for physiological implants. Future work should consider methodological advancement that would allow the collection of physiological data from non-invasive recordings while animals are in more natural settings (i.e., not being held) to evaluate the full range of physiological responsiveness.

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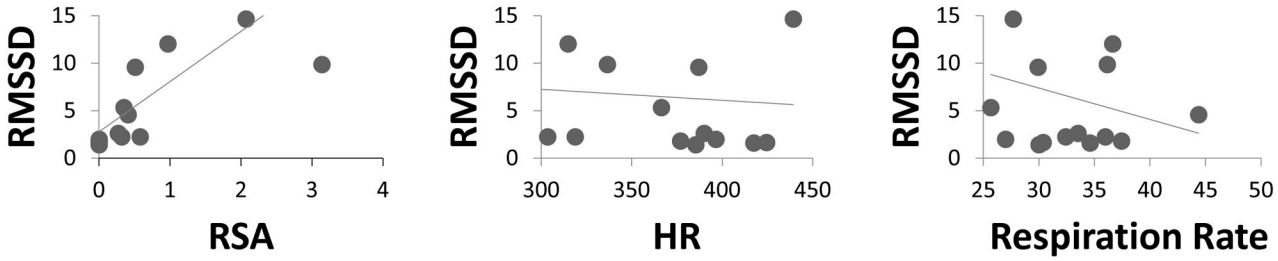

Figure 1.
Relationships between the cardiac variables for the full sample.

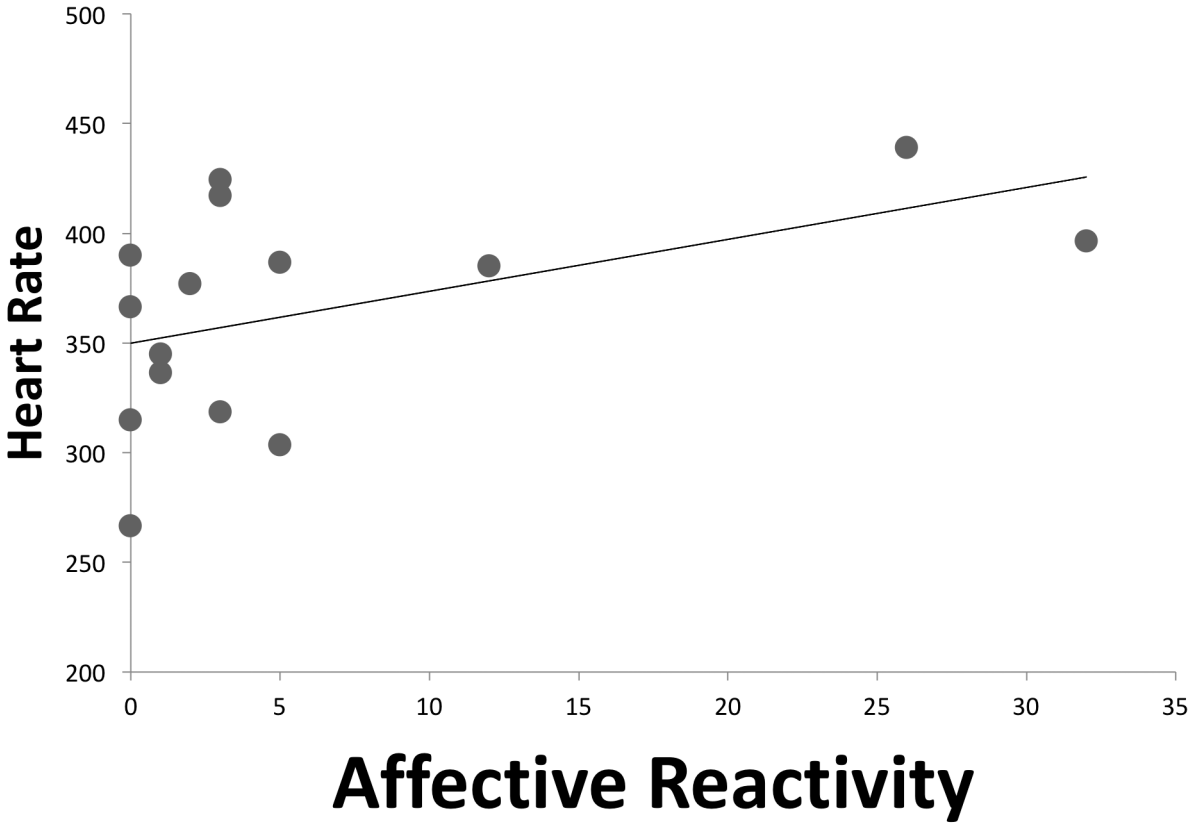

Figure 2.
Relationship between Heart Rate and Affective Reactivity

Table 1:

Behavioral ethogram

Behavioral Category	Behavior	Definition
Affective Reactivity		*Affective responses to cage and/or novel objects*
	Play	Usually manipulating an object, often includes bouncing or quick bursts of activity with the item.
	Attack*	Animal rapidly digs at or bites an object.
	Fearful	Animal is in a submissive pose, often includes ears compressed.
	Twitter	A soft contact vocalization.
	Long Call	A loud, long distance contact call.
	Scratching	Rapid hand movement through fur, not focused grooming.
	Repetitive Motion	Often flipping or pacing.
	Cower	Submissive stance with head and hands tucked close to body.
Interaction		*Animal interacting with cage or novel objects*
	Contact	Animal touches an object.
	Sniff	Animal puts face next to object.
	Attending	Animal is actively looking at a specific object, watching, following.
	Approach	Animal moves toward an object while looking at it, directed movement.
	Attack*	Animal rapidly digs at or bites an object.
Movement		*Quantification of physical position change during testing.*
	Front Top	Animal is in the upper quadrant of the cage, closest to the camera.
	Front Bottom	Animal is in the lower quadrant of the cage, closest to the camera.
	Back Top	Animal is in the upper quadrant of the cage, closest to the rear of the cage.
	Back Bottom	Animal is in the lower quadrant of the cage, closest to the rear of the cage.
	Move	Locomotion

Note: Attack was included both in the Affective Reactivity index as well as the Interaction index because while it represents a physical interaction with the object it is an interaction that has affective significance.

Table 2:

Descriptive statistics of cardiac variables

Measure	Mean	Median	Range	SD
Heart Rate (beats/m)	364.57	376.95	172.47	49.10
Inter-Beat Interval (ms)	168.60	159.52	88.40	24.86
RSA (ln ms^2)	0.70	0.35	3.14	0.95
RMSSD	6.50	2.60	24.45	6.89
Respiration Rate (breaths/m)	32.66	32.41	18.7	5.00
PEP (ms)	21.39	18.15	28.11	11.03

Table 3a.

Stability of Estimates for 15 s and 30 s bins Aggregated to 1 m Across Duration Experiment

	15 s Bins Aggregated to 1 m			30 s Bins Aggregated to 1 m		
Spearman Correlations Between:	**RSA**	**RMSSD**	**HR**	**RSA**	**RMSSD**	**HR**
Minute 1 & *Minute 2*	**0.539** ***(0.047)***	**0.767** ***(0.001)***	**0.833** ***(0.001)***	0.520 *(0.570)*	**0.785** ***(0.001)***	**0.833** ***(0.001)***
Minute 2 & Minute 3	**0.704** ***(0.005)***	**0.820** ***(0.001)***	**0.846** ***(0.001)***	0.508 *(0.064)*	**0.604** ***(0.022)***	**0.846** ***(0.001)***
Minute 1 & Minute 3	0.476 *(0.850)*	**0.705** ***(0.005)***	**0.811** ***(0.001)***	0.427 *(0.128)*	**0.745** ***(0.002)***	**0.811** ***(0.001)***
Intraclass Correlation	**0.810** ***(0.001)***	**0.830** ***(0.001)***	**0.933** ***(0.001)***	**0.805** ***(0.001)***	**0.818** ***(0.001)***	**0.933** ***(0.001)***

Note: Correlation coefficients are presented on the first line of each cell, with p-values in italicized parentheses. Significant correlations are bolded.

Table 3b.

Stability of Estimates for 15 s bins Aggregated to 30 s Across Duration Experiment

Spearman Correlations Between:	RSA	RMSSD
Mean of (0–15 s, 16–30 s) correlated with 0–30 s	**0.911** ***(0.001)***	**0.969** ***(0.001)***
Mean of (31–45 s, 46–60 s) correlated with 31–60 s	**0.853** ***(0.001)***	**0.978** ***(0.001)***
Mean of (61–75 s, 76–90 s) correlated with 61–90 s	**0.907** ***(0.001)***	**0.974** ***(0.001)***
Mean of (91–105 s, 106–120 s) correlated with 91–120 s	**0.994** ***(0.001)***	**0.991** ***(0.001)***
Mean of (121–135 s, 136–150 s) correlated with 121–150 s	**0.856** ***(0.001)***	**0.996** ***(0.001)***
Mean of (151–165 s, 166–180 s) correlated with 151–180 s	**0.413** ***(0.001)***	**0.903** ***(0.001)***
Intraclass Correlation	**0.944** ***(0.001)***	**0.947** ***(0.001)***

Note: Correlation coefficients are presented on the first line of each cell, with p-values are in italicized parentheses. Significant correlations are bolded. Heart rate was perfectly correlated across all analyses and so is not presented here.