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Bolus isoproterenol infusions provide a reliable method for assessing interoceptive awareness

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Abstract

Interoception, defined as the perception of internal body states, plays a central role in classic and contemporary theories of emotion. In particular, deviations from baseline body states have been hypothesized to be integral to the experience of emotion and feeling. Consequently, reliable measurement of interoception is critical to the testing of emotion theories. Heartbeat perception tasks have been considered the standard method for assessing interoceptive awareness, primarily due to their non-invasive nature and technical feasibility. However, these tasks are limited by the fact that above chance group performance rates on heartbeat detection (or the frequency of ‘good detectors’) are rarely higher than 40 percent, meaning that such tasks (as they are typically utilized) do not obtain a measure of interoceptive awareness in the majority of individuals. Here we describe a novel protocol for inducing and assessing a range of deviations in body states via bolus infusions of isoproterenol, a non-selective beta adrenergic agonist. Using a randomized, double-blinded, and placebo-controlled experimental design, we found that bolus isoproterenol infusions elicited rapid and transient increases in heart rate and concomitant ratings of heartbeat and breathing sensations, in a dose-dependent manner. Our protocol revealed changes in interoceptive awareness in all 15 participants tested, thus overcoming a major limitation of heartbeat detection tasks. These findings indicate that bolus isoproterenol infusions provide a reliable method for assessing interoceptive awareness, which sets a foundation for further investigation of the role of interoceptive sensations in the experience of emotion.

Keywords

interoception; isoproterenol; emotion; heartbeat detection; breath

Introduction

Background

Interoceptive sensations occupy a central role in classic and contemporary theories of emotion. In *The Expression of Emotion in Man and Animals*, Charles Darwin (1872) highlighted the involvement of sensations from the viscera in his description of the experience of fear, noting “the heart beats quickly and violently, so that it palpitates or knocks against the ribs” and that “in connection with the disturbed action of the heart the breathing is hurried.” The subsequent highly influential James-Lange theory of emotion, put forth independently by William James (1884) and Carl Lange (1885), posits that signals

originating from the within the body, such as the sensation of the heartbeat and breath, are fundamental for the experience of emotion to the extent that “the feeling of bodily changes as they occur IS the emotion,” and that in the absence of the experience of bodily change all that is left is a “cold and neutral state of intellectual perception” (James, 1884). Since the inception of the James-Lange theory, the role of afferent bodily sensation in emotion has been debated. Against the James-Lange “peripherist” theory, Walter Cannon (1929) and Philip Bard (1928) defended a “centralist” theory of emotion, arguing that the full range of visceral sensations was neither a necessary nor a sufficient condition for the experience of emotion. This was based on observations of intact emotional expression in deafferented cats, intact emotional experience in humans with spinal cord transections, and the absence of genuine reports of emotional experience in humans following sympathetic modulation of visceral sensations with adrenaline (Marañón, 1924). Stanley Schacter and Jerome Singer endorsed a similar view in their “attribution theory” (Reisenzein, 1983; Schacter, 1962), based on studies of epinephrine injections in humans. The theory stated that the subjective perception of physiological arousal, although often a component of the experience of emotion, was not sufficient to elicit specific emotional states. Emotions required an additional cognitive process of attribution of meaning to the perceived physiological response, based on available contextual cues.

Contemporary views continue to highlight the importance of peripheral sensations in the subjective experience of emotion. Beyond support for a general relationship between physiological and subjective arousal it has been suggested that specific patterns of signals within the body, triggered by emotionally competent stimuli and under the control of complex patterns of neural and humoral signaling, can provide a basis for differentiating emotions (Damasio, 1994,1999). More generally, patterns of activity in a network of body-sensitive brain regions are thought to underlie the experience of different emotions (Damasio, 1994,1999,2004;Damasio, et al., 2000). An essential component of feelings, defined as the subjective experience of emotion, would be characterized by the perception of bodily changes mediated by these brain regions (Damasio, 1994,1999;Rainville, Bechara, Naqvi, & Damasio, 2006). Functional neuroimaging studies have provided some preliminary support for this view, demonstrating the activation of viscerosensory and somatosensory brain regions such as the insula, somatosensory cortices and anterior cingulate cortex during the feeling of a wide range of emotions (Blood & Zatorre, 2001;Critchley, Mathias, & Dolan, 2001;Damasio, et al., 2000;Lane, Reiman, Ahern, Schwartz, & Davidson, 1997;Mayberg, et al., 1999;Reiman, et al., 1997). However, the precise role of body-sensitive brain regions in emotion remains a controversial and very much unresolved topic, and other views of emotion have emphasized the roles that sensory brain regions play in encoding and evaluating the reward and punishment values of different stimuli in order to maximize appropriate environmental response selection (Rolls, 1990,2000).

Recent evidence has shed light on some of the neuroanatomical pathways that interoceptive signals use to reach the brain. These include chemosensitive areas of the central nervous system (e.g., area postrema, organum vasculosum of the lamina terminalis, and the subfornical organs), the proprioceptive and vestibular systems, C and A delta fibers of the lamina I spinothalamic pathway, and vagal afferents (Cameron, 2001; Craig, 2002; Saper, 2002). Interoceptive signals are continuously relayed from the body to the brain through key structures in the brainstem such as the nucleus of the solitary tract and the parabrachial nucleus, to the hypothalamus, and through the thalamus (the ventromedial posterior nuclei in particular) where they are mapped and re-represented in several regions of the cerebral cortex, including somatosensory cortices (SI and particularly SII), the insula, the cingulate cortex (particularly the anterior cingulate) and the ventromedial prefrontal cortex (Cameron, 2001; Craig, 2002; Damasio, 2003). It is interesting to note that these structures include the higher-order brain regions that the functional imaging literature has implicated in the

subjective experience of emotion (Blood & Zatorre, 2001; Critchley, Mathias, & Dolan, 2001; Damasio, et al., 2000; Lane, Reiman, Ahern, Schwartz, & Davidson, 1997; Mayberg, et al., 1999; Reiman, et al., 1997). Given the complex, overlapping and controversial functions that are ascribed to these structures, if the role of visceral sensation in the experience of emotion is to be clarified, it is critical that precise and reliable experimental protocols that can manipulate and measure interoceptive awareness are developed. The current study begins to address this pressing need.

Measuring interoceptive awareness

Numerous methods for assessing interoceptive awareness have been described, including gastrointestinal distension (Holzl, Erasmus, & Moltner, 1996; Mayer, Naliboff, & Craig, 2006), adrenergic stimulation (Cameron & Minoshima, 2002; Cameron, Zubieta, Grunhaus, & Minoshima, 2000) and heartbeat perception (Brenner & Kluvitse, 1988; Schandry, 1981; Whitehead, Drescher, & Heiman, 1977). Heartbeat perception has traditionally been the most commonly utilized method, primarily due to the phenomenological relevance of heartbeat sensations to the experience of emotion (Wiens, Mezzacappa, & Katkin, 2000), as well as the technical and non-invasive ease with which this signal can be measured (Jones, 1994; Phillips, Jones, Rieger, & Snell, 1999). Factors modulating awareness of cardiac sensations during the performance of heartbeat perception tasks have been extensively described, including the effects of body mass index (Rouse, Jones, & Jones, 1988), body position (Jones, Jones, Rouse, Scott, & Caldwell, 1987), physical (Barsky, Orav, Delamater, Clancy, & Hartley, 1998; Herbert, Ulbrich, & Schandry, 2007; Schandry, Bestler, & Montoya, 1993) and mental exertion (Eichler & Katkin, 1994), judgments of temporal simultaneity and mechanical sensitivity (Brenner, Liu, & Ring, 1993; Knapp, Ring, & Brenner, 1997; Ring & Brenner, 1992), and heart rate variability (Knapp-Kline & Kline, 2005) (for a comprehensive overview see (Jones, 1994)). Heartbeat perception tasks have also recently been shown to activate the network of brain regions considered necessary for representing and maintaining the internal state of the organism, and important for emotion, including the insula, primary somatosensory cortex and the anterior cingulate cortex (Critchley, Wiens, Rotshtein, Ohman, & Dolan, 2004; Pollatos, Schandry, Auer, & Kaufmann, 2007).

Heartbeat detection and heartbeat tracking tasks are the most commonly utilized methods for assessing perception of heartbeat sensations. During heartbeat detection, participants determine whether or not an exteroceptive stimulus, such as a light or a tone, is simultaneous with their heartbeat sensation (Brenner & Kluvitse, 1988; Schneider, Ring, & Katkin, 1998; Whitehead, Drescher, & Heiman, 1977). Performance is indexed by the number of correct responses reported by the participant (e.g., true positives and true negatives). Participants may then be classified as ‘good heartbeat detectors’ if their performance lies above chance according to the binomial distribution (Katkin, Wiens, & Ohman, 2001; Schneider, Ring, & Katkin, 1998; Wiens & Palmer, 2001). During heartbeat tracking, participants silently count their heartbeats throughout brief, fixed time periods. Performance is indexed by a cardiac perception score, in which the number of counted heartbeats is contrasted with the number of actual heartbeats. Participants are then classified as ‘good heartbeat perceivers’ if their scores fall above a predetermined level (Herbert, Ulbrich, & Schandry, 2007). Heartbeat detection has been the more commonly utilized measure, perhaps because it appears to suffer less from methodological confounds than heartbeat tracking. These include the lack of a statistical measure to evaluate individual performance and the possible influence of a priori knowledge about average heart rate on the rate of counting (Khalsa et al, in press; Phillips, Jones, Rieger, & Snell, 1999; Ring & Brenner, 1996).

In spite of the wealth of accumulated data on heartbeat detection, one curious and scientifically frustrating fact remains: most individuals display chance performance when assessed via heartbeat detection tasks. Across all studies, above chance group performance

rates, or the frequency of ‘good detectors,’ are rarely higher than 40 percent. This has been documented since the inception of methodology for assessing heartbeat detection, regardless of the utilized heartbeat detection method, sample size, participant characteristics or research question (Brener & Kluvitse, 1988; Brener, Liu, & Ring, 1993; Eichler & Katkin, 1994; Jones, Jones, Rouse, Scott, & Caldwell, 1987; Jones, O’Leary, & Pipkin, 1984; Knapp, Ring, & Brener, 1997; Knapp-Kline & Kline, 2005; Ring & Brener, 1992; Rouse, Jones, & Jones, 1988; Schneider, Ring, & Katkin, 1998; Whitehead, Drescher, & Heiman, 1977; Wiens & Palmer, 2001; Yates, Jones, Marie, & Hogben, 1985; Khalsa et al, in press). Furthermore, it has been noted that participants frequently report they were simply guessing during the heartbeat detection task (Wiens, 2005). Low rates of awareness are also congruent with results from heartbeat tracking tasks, where it is common for investigators to have to screen and exclude a much larger number of “poor perceivers” in order to obtain equal numbers of good and poor perceivers (Pollatos, Herbert, Matthias, & Schandry, 2007; Pollatos, Kirsch, & Schandry, 2005). A clue as to why this may be comes from the fact that most studies of heartbeat perception occur under conditions of physiological rest, when there are few deviations from the baseline state of the body. Indeed, heartbeat perception accuracy has been reported to increase when deviations from baseline body states occur such as during exercise or stress (Jones & Hollandsworth, 1981; Schandry, 1980; Schandry, Bestler, & Montoya, 1993). This suggests that there is an inherent limitation in the ability to detect the heartbeat at rest, and that this limitation may be overcome during conditions of increased physiological arousal. If the role of interoceptive sensation in the experience of emotion is to be clarified, additional approaches must be developed that can reliably manipulate and measure interoceptive awareness in most—ideally, perhaps even all—participants.

Adrenergic stimulation represents one promising solution to the limitations imposed by conducting heartbeat perception tasks at rest. This approach provides the ability to reversibly modulate the bodily state of the organism above baseline levels, in a sympathomimetic manner resembling a subset of the physiological changes known to occur during emotional states. Although adrenergic stimulants have been utilized in influential emotion research during the past century (Marañón, 1924; Reisenzein, 1983; Schacter, 1962), no standard protocols exist for assessing subjective awareness of the interoceptive states produced by these agents. Thus, although Marañón (1924) and Schacter & Singer (1962) manipulated the state of the body using epinephrine injections, both relied on open ended descriptions and a basic retrospective assessment of the interoceptive sensations induced by these infusions. Furthermore, because Schacter & Singer (1962) and others (Marshall, 1979; Mezzacappa, 1999) have relied on subcutaneous injections of epinephrine, the time course during which changes in body state were elicited varied between 10 minutes to an hour. Given the transient nature of many emotional states (Ben-Ze’ev, 2000; Davidson, 2003; Hutcherson, et al., 2005) and the interoceptive sensations associated with them, alternative techniques for inducing similar changes in interoceptive awareness are needed if the nature of the interactions between emotional experience and interoception are to be clarified.

One promising protocol that has emerged consists of a standardized isoproterenol sensitivity test. This involves the graded administration of isoproterenol, a non selective beta adrenergic agonist (Arnold & McDevitt, 1983; Cleaveland, Rangno, & Shand, 1972; Contrada, Dimsdale, Levy, & Weiss, 1991; George, Conolly, Fenyvesi, Briant, & Dollery, 1972; Martinsson, Lindvall, Melcher, & Hjendahl, 1989; Mills, Dimsdale, Ancoli-Israel, Clausen, & Loreda, 1998; Yu, Kang, Ziegler, Mills, & Dimsdale, 2007). When administered intravenously, isoproterenol primarily results in rapid elevations in heart rate and contractility, relaxation of bronchial smooth muscle, and reductions in diastolic blood pressure. The pharmacological effects of isoproterenol are transient, owing to a short half-life in the blood (Conolly, et al., 1972), providing an opportunity for repeated administrations with reproducible effects within a single experimental session (Martinsson,

Lindvall, Melcher, & Hjemdahl, 1989). Furthermore, since isoproterenol is believed to only minimally cross the blood brain barrier (Borges, Sarmiento, & Azevedo, 1999; Murphy & Johanson, 1985; Olesen, Hougaard, & Hertz, 1978), it is unlikely that the effects of isoproterenol administration result directly in changes in brain activity. This presents a unique opportunity to examine the effects of stimulation restricted to afferent sensory nerve fibers on interoceptive awareness and emotional experience.

Although it has been known for some time that the pharmacological effects of isoproterenol elicit changes in cardiac and respiratory sensations (Cleaveland, Rangno, & Shand, 1972; George, Conolly, Fenyvesi, Briant, & Dollery, 1972), few studies have specifically examined the nature of these changes. In a recent Positron Emission Tomography study, Cameron and Minoshima (2002) administered a continuous infusion of either isoproterenol to maintain a heart rate of 120 beats per minute (bpm) in one group, or a saline placebo infusion for 30 minutes in another group. Using a single blinded design, participants were asked to rate their awareness of cardiac, respiratory, and affective symptoms, before and after completion of the infusion period. In both groups isoproterenol or saline administration was preceded by infusion of a fluorodeoxyglucose radiotracer in order to assess isoproterenol induced changes in regional cerebral glucose metabolism. Participants in the isoproterenol but not the saline infusion condition reported an increase in awareness of cardiac and respiratory sensations, as well as an increase in symptoms of physical anxiety, mental anxiety and distress. These changes in interoceptive sensations were concomitant with regional increases in glucose metabolism in brain regions including the right insula, left SI, and dorsal cingulate cortex. This enhancement of metabolism in interoceptive brain regions further illustrates the validity of isoproterenol as a tool for measuring interoceptive awareness, and the reported changes in affective state also emphasize its relevance for emotion research.

In the Cameron & Minoshima (2002) paradigm, isoproterenol was titrated to maintain a constant and elevated level of body arousal. However, since perceived body states during the experience of emotion are not static, a more desirable approach involves assessing interoceptive awareness produced by various levels of transient bodily changes, as are found in standard isoproterenol sensitivity tests. In addition, since the experience of interoceptive sensations is continuous, new methodologies capable of capturing ongoing, moment-to-moment changes in perceived body state would seem to be highly desirable.

The current study

In response to the limitations of conventional interoceptive awareness tasks described above, and based on the promise of the isoproterenol approach, we developed a novel protocol for manipulating and measuring interoceptive awareness. This protocol was based on the standardized isoproterenol sensitivity test, and involved multiple bolus administrations of isoproterenol in a randomized, double blinded, and placebo controlled manner. We report the results of this study in the current article. The ultimate goal of this work is to provide a foundation for further investigations of the interactions between interoception and emotion.

In order to elicit a full range of changes in interoceptive awareness, we chose doses that were likely to be below and above thresholds for detection in each participant, based on preliminary testing. As a first pass measure of these changes in interoceptive awareness, we employed retrospective ratings of interoceptive sensations in a manner similar to Cameron & Minoshima (2002). These retrospective ratings were supplemented with continuous dial ratings of the intensity of interoceptive sensations experienced throughout each infusion, a form of continual self monitoring that helps minimize demand characteristics, response biases and potential memory confounds associated with retrospective ratings (Craske & Tsao, 1999; Hutcherson, et al., 2005). Finally, since there may be confounds associated with

asking participants to explicitly rate the experience of subconscious stimuli (Cleeremans, Destrebecqz, & Boyder, 1998), we employed a post infusion wagering task requiring participants to place imaginary wagers on whether they had received isoproterenol or saline (placebo). This measure was based on post decision wagering, a newly developed measure purported to be an intuitive and direct measure of awareness (Persaud, McLeod, & Cowey, 2007).

We hypothesized that bolus infusions of isoproterenol would result in dose-dependent increases in retrospective ratings of heartbeat and breathing sensations, as indexed by intensity ratings of heartbeat and breathing sensations and post infusion wagering. We also hypothesized that there would be a dose-dependent correlation between the continuous subjective ratings of isoproterenol-related interoceptive sensations and the objective bodily response to isoproterenol, as indexed by the change in heart rate. Critically, we also explored whether the aforementioned changes in interoceptive awareness would be detectable in the majority of participants, at least at higher doses, which would overcome a major limitation of extant heartbeat perception tasks.

Methods

Participants

15 healthy individuals (10 men, 5 women) participated in the study (see table 1 for complete demographics). All participants were screened for the presence of any neurological, psychiatric, cardiac or respiratory disease during a detailed phone interview, and were excluded if they reported a history of disease in any of these categories. None of the study participants were smokers, and none of the women took oral contraceptives or were pregnant, as assessed via urine pregnancy test. Each participant demonstrated a normal 12 lead electrocardiogram (EKG), as assessed by a board certified cardiologist or neurologist.

Tasks

Participants rated the experience of heartbeat and breathing sensations during and immediately following bolus infusions of isoproterenol and normal saline. Participants were told they would be receiving both isoproterenol and saline infusions, and were informed what the isoproterenol sensations might feel like (e.g., “you may notice your heart beating faster, and/or may feel an increase in your breathing sensations”). They were not informed when they would be receiving each agent, but were verbally notified of the beginning of each infusion (e.g., “infusion starting”). Each infusion period lasted approximately 2 minutes. During each period participants were instructed to pay attention to their heartbeat and breathing sensations, and to rotate a dial to indicate their ongoing experience of the overall intensity of these body sensations. The dial could range from 0 (“normal, i.e., no change in intensity”) to 10 (“most ever”). The dial was always set to zero at the beginning of each infusion, and participants were specifically instructed to keep the dial at zero if they felt they did not notice any increase in the intensity of heartbeat and breathing sensations above baseline. After each infusion, participants rated the intensity of heartbeat and breathing sensations they had experienced during the prior infusion period. In particular, they were instructed to rate via questionnaire the overall intensity of heartbeat and breathing sensations they had experienced during each infusion, from 0 (“normal, i.e., no change in intensity”) to 10 (“most ever”), in the manner described by Cameron & Minoshima (2002).

Participants were then instructed to accurately trace on a manikin template the locations where they felt heartbeat sensations within their own body. Next, participants were instructed to rate the intensity of physical anxiety, mental anxiety, and distress experienced during each infusion using the same 0 to 10 rating scale. In an effort to calibrate each

participant's understanding of the affective terminology employed, prior to infusion administration physical anxiety was operationalized as the "the bodily sensations you associate with the experience of being anxious," mental anxiety was operationalized as "worry, for example, the kind you might experience if you were running late for an important appointment," and distress was operationalized as "alarm, for example, the kind you might experience if you realized your house was on fire and needed to escape".¹ Finally, participants were asked to place imaginary wagers on whether their heartbeat or breathing had changed during the preceding infusion period (e.g., "if you were going to bet that there was a change in your heartbeat [or breathing] induced by what you received through the IV, how much would you be willing to wager?") (Persaud, McLeod, & Cowey, 2007). Any amount between 0 and 20 dollars could be wagered, and participants were instructed to bet 0 dollars if they were confident they had received a saline infusion.

Infusion protocol

Participants received 3 sets of isoproterenol infusions. The first two sets of infusions comprised a standard isoproterenol infusion protocol, which consisted of sequentially increasing isoproterenol doses of 0.1, 0.5, 1.0, 2.0 and 4.0 micrograms (mcg) (Cleaveland, Rangno, & Shand, 1972; Contrada, Dimsdale, Levy, & Weiss, 1991; Martinsson, Lindvall, Melcher, & Hjendahl, 1989; Mills, Dimsdale, Ancoli-Israel, Clausen, & Lored, 1998; Yu, Kang, Ziegler, Mills, & Dimsdale, 2007). Participants were not instructed to rate the experience of interoceptive sensations during these infusions. These protocols were used to establish the chronotropic dose 25 (CD25), or the isoproterenol dose necessary to increase the participant's heart rate by 25 beats per minute above baseline. The CD25 is a commonly reported measure of beta adrenergic receptor sensitivity and was calculated by extrapolation from the slope of a linear regression at each individual's isoproterenol induced heart rate response (mean heart rate response at each isoproterenol dose minus baseline heart rate) (Arnold & McDevitt, 1983; Cleaveland, Rangno, & Shand, 1972; Mills, Dimsdale, Ancoli-Israel, Clausen, & Lored, 1998; Yu, Kang, Ziegler, Mills, & Dimsdale, 2007). This administration order also ensured that each participant was familiar with the sensations elicited by isoproterenol prior to collection of interoceptive ratings. The third set of infusions comprised the interoceptive rating condition, which consisted of a total of 12 randomized infusions: 6 normal saline and 6 isoproterenol (0.1, 0.25, 0.5, 0.75, 1.0 and 2.0 mcg). The decision not to include a 4.0 mcg dose in the interoceptive rating condition was based on preliminary testing with a different sample of participants, in which we found that all participants reported changes in awareness at the 2.0 mcg dose. We chose instead to replace the 4 mcg dose with a lower dose (0.25 mcg), in order to more effectively determine the minimum dose that would result in changes in interoceptive awareness. The CD25 for this third set of infusions was also calculated, for comparison with the first two sets of infusions. All infusions were administered a minimum of 3.5 minutes apart.

Infusion delivery

Each infusion (isoproterenol and saline) consisted of two 3 milliliter (ml) bolus infusions delivered sequentially through an intravenous catheter. During isoproterenol infusions, a 3 ml bolus containing the specified dose was delivered, immediately followed by a 3 ml bolus of saline to flush the line. During saline infusions, a 3 ml bolus of saline was delivered, immediately followed by an additional 3 ml bolus of saline. Both bolus volumes were administered in entirety within a 15 second period by a nurse from the General Clinical Research Center. This method of delivery minimized the participant's ability to use external

¹These specific examples were chosen to reflect affective experiences that most individuals were either likely to have encountered in their own lives or could imagine as a realistic possibility.

cues to distinguish between the different infusion types, and ensured rapid and standardized systemic introduction of isoproterenol.

Procedure

The study involved one visit, which always started between 7 and 8am in the General Clinical Research Center (GCRC) at the University of Iowa. After completing the consent process participants filled out several questionnaires to assess demographics such as age, education, current levels of anxiety (Beck, 1990), depression (Beck, 1993), and positive and negative affective experience (Watson, 1988). Afterwards, a nurse measured each participant's height and weight, and female participants completed a urine pregnancy screen. The nurse then placed a 22 gauge intravenous catheter into the participant's non dominant dorsal hand vein, and administered a 12 lead EKG. A physician evaluated the EKG, and the experiment proceeded only if the EKG was considered normal (all recruited participants displayed normal EKGs). The participant was led to a quiet room, seated in a comfortable chair, and was attached to leads for measuring heart rate (lead II EKG), respiratory rate (thoracic respiratory belt) and skin conductance response (non dominant thenar and hypothenar eminence). At this point the participant's non dominant hand was placed outstretched on a pillow at chest level. A curtain was positioned with the participant on one side and the nurse and the experimenter on the other side, to prevent the participant from viewing the preparation and administration of each infusion. The nurse then measured the participant's blood pressure and began the isoproterenol infusion protocol. Participants were instructed not to recline in the chair during each infusion period, in order to prevent them from using the back of the chair as an external source to help them detect heartbeat sensations. The entire testing session lasted approximately four hours. This study was approved by the GCRC Advisory Committee and the Institutional Review Board of the University of Iowa, and all participants provided informed consent prior to participation.

Psychophysiological measures—All physiological data including heart rate were recorded continuously during all infusions with an MP100 acquisition unit (Biopac Systems, Inc) at a sampling rate of 200 Hertz. Dial ratings were collected with a custom built dial that consisted of a rotating potentiometer with a continuous rating scale ranging from 0.000 to 5.000 Volts. The average heart rate response during each infusion was calculated across a 120 second interval immediately following the onset of each infusion. The average heart rate response was obtained by subtracting the average heart rate during the 30 second post infusion window (before isoproterenol induced heart rate changes had occurred) from the average heart rate during the subsequent 90 second window (when the isoproterenol induced heart rate changes were most likely to occur). These windows were carefully chosen to coincide with the typical delays observed in the onset of isoproterenol induced heart rate changes due to the slow rate of venous drainage to the heart (Arnold & McDevitt, 1983; Cleaveland, Rangno, & Shand, 1972; Contrada, Dimsdale, Levy, & Weiss, 1991; Mills, Dimsdale, Ancoli-Israel, Clausen, & Lored, 1998; Yu, Kang, Ziegler, Mills, & Dimsdale, 2007). Peak heart rate responses were also calculated for each participant, defined as the maximum heart rate change occurring within a five second interval around the maximum heart rate change observed (during the 90 second infusion window) relative to the average heart rate during the 30 second baseline window. All artifacts affecting the instantaneous heart rate waveform (e.g., movement related, or due to premature ventricular contractions) were manually identified and removed.

Cross correlations for each participant were calculated from mean centered dial ratings and instantaneous heart rate changes occurring over the two minute interval following the onset of each infusion. This interval included the 30 second window following the infusion onset when isoproterenol induced HR changes had not yet occurred, as well as the subsequent 90

second window when isoproterenol induced heart rate changes were most likely. Dial ratings and instantaneous heart rate changes for each dose were mean centered by subtracting the 120 second mean for each infusion interval from each time point within that interval.

Data analysis—Single factor, repeated measures ANOVAs were performed with dose of isoproterenol as the independent factor and isoproterenol induced change scores as the dependent factor. Change scores were calculated by subtracting the mean value of all six saline responses from each isoproterenol response (Cleaveland, Rangno, & Shand, 1972; Contrada, Dimsdale, Levy, & Weiss, 1991). This approach provided a robust estimate of the baseline and enabled a sensitive determination of the effect of isoproterenol doses on deviations from baseline for each measure. If an overall significant effect of isoproterenol dose was detected, post hoc t-tests were performed using Tukey's HSD method to determine significant differences between the pairwise comparisons ($p = .05$ level). All measures were assessed for violations of the sphericity assumption, and when violated, were corrected with the Huynh-Feldt method. In these instances the corrected p values are reported, along with the Huynh-Feldt epsilon (ϵ) correction. Finally, Pearson's correlations were calculated to determine if relationships existed between isoproterenol sensitivity (CD25 and dose-specific heart rate responses), interoceptive ratings, and demographic factors such as age, BMI, reported levels of anxiety, depression and positive and negative affect.

Results

Heart rate response to isoproterenol and saline infusions

A repeated measures ANOVA revealed a significant effect of isoproterenol on the mean heart rate response $F(5, 70) = 18.23, p < .0001, \eta_p^2 = .57, \epsilon = .531$, indicating that isoproterenol infusions elicited increases in heart rate (fig 1A). Post hoc testing revealed that the mean heart rate response significantly increased at the three highest doses of isoproterenol (0.75, 1.0 and 2.0 mcg). A repeated measures ANOVA also revealed a significant effect of isoproterenol on the peak heart rate response $F(5, 70) = 22.08, p < .0001, \eta_p^2 = .61$. Post hoc testing revealed that the peak heart rate response significantly increased at the four highest doses (0.5, 0.75, 1.0 and 2.0 mcg). The mean CD25 values obtained during the isoproterenol sensitivity tests and during the interoceptive ratings are listed in table 1. No significant differences in the CD25 values were observed across the three conditions $F(2, 28) = .09, p = .92$, suggesting that habituation to isoproterenol did not occur with repeated administration. The group's average heart rate during all saline infusions was 67.8 ± 11.3 bpm.

Retrospective interoceptive awareness ratings

A repeated measures ANOVA revealed a significant effect of isoproterenol on retrospective ratings of the overall intensity of heartbeat sensations $F(5, 70) = 20.1, p < .0001, \eta_p^2 = .59, \epsilon = .44$, indicating that isoproterenol infusions elicited greater changes in awareness of heartbeat sensations than saline (fig 1B). Post hoc testing revealed that increased ratings of heartbeat sensations occurred at the three highest doses (0.75, 1.0 and 2.0 mcg).

A repeated measures ANOVA revealed a significant effect of isoproterenol on retrospective ratings of the overall intensity of breathing sensations $F(5, 70) = 10.1, p < .0002, \eta_p^2 = .42, \epsilon = .496$, indicating that isoproterenol infusions elicited greater changes in awareness of breathing sensations than saline (fig 1E). Post hoc testing revealed that increased ratings of breathing sensations occurred at the three highest doses (0.75, 1.0 and 2.0 mcg).

Post infusion wagering

A repeated measures ANOVA revealed a significant effect of isoproterenol on post infusion wagering on heartbeat change $F(5, 70) = 19.37, p < .0001, \eta_p^2 = .58$, indicating that isoproterenol infusions elicited greater changes in wagering amounts than saline (fig 1C). Post hoc testing revealed that increased wagering on heartbeat change occurred at the three highest doses (0.75, 1.0 and 2.0 mcg).

A repeated measures ANOVA revealed a significant effect of isoproterenol on post infusion wagering on breathing change $F(5, 70) = 21.2, p < .0001, \eta_p^2 = .60$, indicating that isoproterenol infusions elicited greater changes in wagering amounts than saline (fig 1F). Post hoc testing revealed that increased wagering on breathing change also occurred at the three highest doses (0.75, 1.0 and 2.0 mcg).

Online dial ratings

Figure 2A shows the observed mean heart rate and corresponding mean dial ratings produced by all participants throughout each 120 second infusion interval, for all doses of isoproterenol. In this figure, dial ratings have been normalized by scaling the dial rating amplitude for each dose to each participant's maximum heart rate change observed during the 2.0 mcg dose, according to the following formula: normalized instantaneous dial rating = instantaneous heart rate at initial sample + maximum heart rate change at 2.0 mcg $\times$ (instantaneous dial rating/5.000). As a result, possible dial rating amplitudes range from a minimum of 0 to a maximum of the peak heart rate observed during the 2.0 mcg dose.

A repeated measures ANOVA revealed a significant effect of isoproterenol on the zero order cross correlation $F(5, 70) = 3.85, p = .004, \eta_p^2 = .22$, indicating that participants generated greater zero lag cross correlations at increasing doses of isoproterenol (fig 2B). Post hoc testing revealed that participants generated increased zero order cross correlations only at the highest dose (2.0 mcg). A repeated measures ANOVA revealed a significant effect of isoproterenol on the maximum cross correlation $F(5, 70) = 14.85, p < .0001, \eta_p^2 = .52$, indicating that participants generated greater maximum cross correlations (irrespective of lag) at increasing doses of isoproterenol (fig 2C). Post hoc testing this time revealed that participants generated increased maximum cross correlations at the four highest doses (0.5, 0.75, 1.0 and 2.0 mcg). A secondary analysis examined whether the absolute value of the lag times obtained at the maximum cross correlation differed for the isoproterenol infusions. A repeated measures ANOVA revealed a significant effect of isoproterenol on the absolute lag times $F(5, 70) = 2.46, p = .041, \eta_p^2 = .15$, indicating that participants generated lower lag times at increasing doses of isoproterenol (fig 2D). Post hoc testing revealed participants generated lower lag times only at the highest dose (2.0 mcg).

Percentage of participants reporting increased interoceptive sensations

Examination of the individual online dial ratings revealed that increasing numbers of participants perceived increases in heartbeat and breathing sensations at increasing doses (fig 3A). Not surprisingly, the lowest increases in sensation were reported during the saline infusions (30% of all saline trials administered). A minority of participants perceived increased interoceptive sensations at the two lower doses (0.1, 0.25 mcg) whereas a majority of participants perceived increased interoceptive sensations at the four highest doses (0.5, 0.75, 1.0 and 2.0 mcg). Critically, every single participant (15/15) perceived increases in sensation at the highest dose (2.0 mcg), and the peak sensation ratings at this dose were highly correlated with the observed peak heart rate changes ($r = .746, p = .001$) (fig 3B).

Affective measures

A repeated measures ANOVA revealed a significant effect of isoproterenol on ratings of physical anxiety $F(5, 70) = 6.28, p = .01, \eta_p^2 = .31, \varepsilon = .328$. Post hoc testing revealed that increased ratings of physical anxiety occurred only at the highest dose (2.0 mcg). There were no significant increases in the ratings of mental anxiety $F(5, 70) = 2.97, p < .10, \varepsilon = .248$, or distress $F(5, 70) = 2.15, p < .15, \varepsilon = .314$ (fig 1D). There were no significant correlations between the CD25 values obtained during any of the isoproterenol administrations and age, BMI, level of anxiety (assessed prior to infusion administration via Beck Anxiety Inventory) depression (Beck Depression Inventory), or positive and negative affect.

Locations of heartbeat sensations

Overlap maps of the locations of perceived heartbeat sensations at each infusion are plotted for all participants in figure 4. These maps indicate that as the dose of isoproterenol increased, a greater number of participants perceived heartbeat sensations in the anterior chest, particularly in the lower left region. At the two highest doses (1.0 and 2.0 mcg), the majority of participants reported feeling the heartbeat in this location. There was also variability in the localization of the heartbeat sensation, with some participants reporting feeling the heartbeat sensation in the head, neck, the center of the belly or arms. The overlap map for the average of all saline infusions indicated that a minority of the participants also perceived heartbeat sensations during the saline infusions, primarily in the lower left chest.

Discussion

As expected, bolus isoproterenol infusions elicited rapid and transient dose-dependent increases in heart rate. These increases were evident across the mean as well as peak heart rate responses. Significant increases in mean heart rate were observed at the three highest doses (0.75, 1.0 and 2.0 mcg), whereas increases in peak heart rate were observed at the four highest doses (0.5, 0.75, 1.0 and 2.0 mcg). The calculated CD25 values from the isoproterenol sensitivity tests and the interoceptive rating condition suggest that the levels of bodily change observed in the current study are similar to those commonly reported in the literature (Mills, Dimsdale, Ancoli-Israel, Clausen, & Lored, 1998; Yu, Kang, Ziegler, Mills, & Dimsdale, 2007).

Concomitant with these changes in peripheral body state, bolus isoproterenol infusions elicited changes in cardiac and respiratory sensations. Increases in interoceptive awareness were observed at increasing doses of isoproterenol, as indexed by retrospective ratings, post infusion wagering, and continuous dial ratings. Interestingly, these increases in interoceptive awareness were indexed to a somewhat different extent by each rating method. Retrospective ratings of interoceptive sensations and post infusion wagering indicated that increased awareness of both heartbeat and breathing sensations occurred at the three highest doses (0.75, 1.0 and 2.0 mcg), perhaps suggesting that these two tasks draw upon a similar type of information when utilized in a retrospective fashion. In contrast, after accounting for the lag time, the cross correlations measured via continuous dial ratings indicated that increases in interoceptive awareness occurred at the four highest doses (0.5, 0.75, 1.0, and 2.0 mcg). Since heart rate changes were observed at the four highest doses of isoproterenol, it appears that the continuous dial ratings provided a more sensitive measure for detecting changes in interoceptive awareness than retrospective ratings. This is understandable given that the dial rating method provides a higher resolution scale for reporting momentary and/or subtle changes in interoceptive sensation, over a more nuanced window of time (e.g., continuous online versus single retrospective). Nevertheless, from the observed lag times it also appears that there are significant delays between the objective changes in body state and

subjective perceptions of these changes, even for doses that readily elicit increases in interoceptive awareness. These delays are consistent with findings from other modalities of visceral sensation such as gastrointestinal distension, in which the time course and quality of visceral sensations correlate imperfectly with visceral stimulation (Aziz, et al., 2000; Cervero, 1985; Holzl, Erasmus, & Moltner, 1996). Identifying the neurophysiological mechanisms underlying these multimodal delays in awareness represents an important area for further investigation, one that may yield critical insights into the neural basis of interoceptive awareness.

At the four highest doses of isoproterenol (0.5, 0.75, 1.0 and 2.0 mcg) the majority of participants perceived increased interoceptive sensations, and at the highest dose all participants reported increases in interoceptive sensations. In addition, the degree to which these sensations were perceived was highly correlated with the degree of observed heart rate change at the highest dose. These findings indicate that our method has overcome a major limitation of previous methods, for example, the widely reported finding of less than 40% accuracy rates for resting heartbeat detection tasks. Specifically, our method appears to provide the capacity to reliably manipulate and measure awareness of interoceptive sensations in most if not all participants. Accordingly, instead of examining differences between good and bad heartbeat detectors (or perceivers), the relationship between interoceptive sensations and emotion (or any other variable under investigation) might be measurable in every participant, at varying levels of interoceptive awareness. The fact that the intensity of subjective sensations was highly correlated with the degree of observed heart rate changes also speaks to the efficacy of the current approach, although future studies are needed to examine how performance on this protocol compares with performance on standard heartbeat perception tasks. For example, it would be interesting to examine whether good heartbeat detectors are more aware of isoproterenol induced heartbeat sensations (e.g., generate greater retrospective ratings at lower doses than non detectors and/or greater cross correlations). An additional benefit of the present method relates to improved ecological validity: rather than abstractly comparing heartbeat sensations to tones or counting heartbeats, participants simply indicate the degree to which their interoceptive sensations are changing in real time. Furthermore, they do so in a manner that shares closer phenomenological proximity to the experience of naturally occurring changes in levels of physiological arousal that, notably, also arise within the context of emotional experience.

Isoproterenol doses elicited a small increase in retrospective ratings of physical anxiety but did not elicit increased ratings of mental anxiety or distress, indicating that the effects of the bolus isoproterenol infusions were disproportionately restricted to experiences of physical body sensations. The observed pattern of findings is somewhat different from the ratings reported by Cameron & Minoshima (2002), who found that a continuous 30 minute infusion of isoproterenol titrated to a heart rate of 120 bpm resulted in increased ratings of anxiety and distress. Since the current study utilized lower doses of isoproterenol and in a bolus format, few participants' heart rates ever reached 120 bpm (peak heart rate reached 120 bpm for only 2 of the 15 participants). As a result, participants in the current study experienced smaller changes in arousal and for briefer periods of time. However, these changes were an intended feature of the design: they were aimed at better mimicking the transient aspect of emotions, and were in light of the fact that extreme changes in arousal are not required for an experience to be reported as emotional (Ben-Ze'ev, 2000; Davidson, 2003; Hutcherson, et al., 2005). In the Cameron & Minoshima (2002) study, the duration and magnitude of the heart rate increase was such that participants may have generated anxiety about being in this state for so long, with potential distress due to the lack of controllability over such an extended elevation in the state of arousal. Thus it is possible that the reported anxiety might have not been the direct reflection of the physiological activation (as would be predicted in a James-Lange theoretical framework), but rather a secondary development of an emotional

state. It is interesting to note that in the current study, participants only perceived the intensity of interoceptive sensations as moderate at the maximum dose (on average not exceeding 5 on a scale of 10). Future studies could address whether reports of anxiety or other emotions can be induced at higher doses approximating or even exceeding the heart rate changes observed by Cameron & Minoshima (2002).

In a broader context, the fact that transient changes in peripheral arousal were not sufficient to induce negatively valenced affective states would appear to argue against a literal interpretation of the James-Lange theory. However, the current study was not specifically designed to tackle this issue, and thus our comments here should be taken as speculative. We only mention it as a possibility because the current method provides a powerful tool for evaluating the roles that interoceptive awareness have been proposed to play in the experience of emotional states. Thus, one benefit of this method could allow for investigations of the degree to which the elicited patterns of cardiorespiratory responses are capable of inducing primary and secondary emotions, as suggested by the James-Lange theory. Similarly, by combining the current method with adequate manipulations of emotional context (a la Schacter & Singer, 1962), novel insights could be generated that refine our understanding of the relative influences of interoceptive and cognitive states on the subjective experience of emotion. Yet another viable area of inquiry relates to the extent to which interoceptive sensations are at all relevant for emotional states (a la Rolls, 2000; i.e., whether they are a necessary component or are merely a downstream consequence of emotional processing). Beyond basic emotion research, this method could also be used to clarify other putative influences of peripheral body states on cognition, such as the contribution of 'gut feelings' to complex decision making (a la the Somatic Marker Hypothesis of Damasio, 1996).

Nevertheless, at the most basic level, the current method provides a framework for studying the phenomenology of, and mechanisms underlying interoceptive awareness. For instance, overlap maps of the location of perceived heartbeat sensations indicated that heartbeat sensations induced by isoproterenol were most commonly experienced in the lower left side of the anterior chest, in a region roughly corresponding to the point of maximum impulse (or PMI). The PMI is considered the location where the heart rotates, moves forward and strikes against the chest wall during systole, and is a physical exam sign routinely utilized by physicians to help them determine if an individual has an enlarged heart (in which case the location of the PMI is shifted). However, heartbeat sensations were also commonly perceived in several other body locations including the head, neck, abdomen and arms. Heartbeat sensations have been localized to many of these same regions in previous studies of heartbeat detection (Jones, 1994; Jones, Jones, Rouse, Scott, & Caldwell, 1987; Ring & Brener, 1992; Khalsa et al, unpublished data), indicating that the observed variability in the present study is reliable. This raises the question of which neural pathways within the body mediate awareness of heartbeat sensations. Possible mechanisms include signal transmission via sensory pathways from receptors in the heart, such as low-threshold mechanosensitive endings on vagal afferent fibers in the atria and venoatrial junction, or mechanosensitive C-fibers in the ventricles (Longhurst, 2004; Malliani, 1986). Another possibility includes intra-thoracic detection of the force generated by the heart beat on the walls of the great vessels (e.g., via baroreceptors) and in surrounding mechanosensitive thoracic tissues (Eichler & Katkin, 1994; Schandry, Bestler, & Montoya, 1993). Yet another possibility includes transmission by cutaneous (dermal and epidermal) mechanosensitive fibers overlying larger arteries. Each of the aforementioned peripheral pathways project to different regions in the brainstem and cerebral cortex (e.g., insula versus primary or secondary somatosensory cortex), and thus have implications for whether heartbeat sensations should be categorized as visceral sensation, cutaneous sensation, or both. An examination of the overlaps from the present study indicates that several of the reported body locations, such as the neck, belly

and head share close proximity with major arteries (e.g., common carotid, abdominal aorta and external carotid arteries respectively). This, in combination with the knowledge that individuals with a lower body mass index are better at detecting heartbeat sensations (Rouse, Jones, & Jones, 1988), suggests that receptors in the skin may play a role in the apprehension of heartbeat sensations. Indeed, Knapp, Ring, & Brener (1997) reported that vibrotactile sensitivity in the finger accounted for a portion of the variance in a heartbeat detection task, and based on this finding suggested that heartbeat sensations might be mediated via Pacinian corpuscles. Although the present findings are not capable of distinguishing whether the skin or deeper structures in the viscera were mediating heartbeat sensations, it seems plausible that a combination of both is occurring. For example, it is possible that structures within the thoracic cavity relay heartbeat sensations localized to the chest, whereas receptors in the skin may transmit heartbeat sensations experienced in other body locations such as the belly, neck and head.

Another important question is which neuroanatomical structures within the brain mediate awareness of heartbeat sensations. Based on the observed variability in the location of heartbeat sensations and the available sensory pathways within the body, it seems plausible that a combination of visceral and somatosensory structures contribute to the perception of heartbeat sensations. This notion is supported by the findings of Cameron & Minoshima (2002), who reported increased regional metabolism of both the insula and midline (truncal) primary somatosensory cortex in participants receiving isoproterenol infusions. However, it is still unclear whether these brain regions truly mediate awareness of heartbeat sensations since functional neuroimaging studies are not capable of determining whether brain regions are required for the ability to experience the sensation of the heartbeat. For example, even though both brain regions show greater metabolism during the experience of heartbeat sensations, it is possible that only one of these is important for the ability feel the heartbeat, or that the two regions provide differential contributions to the heartbeat sensation. These questions could be addressed by adapting the current protocol to human lesion studies, for example, by studying the experience of heartbeat sensations in individuals with damage to insular or somatosensory cortex.

There are several limitations associated with the current study. Since isoproterenol induces inotropic as well as chronotropic changes in cardiac function, one limitation of the cross correlation method relates to the isolated use of heart rate change for calculating the interoceptive cross correlations. We do not consider this to be a major limitation, though, because cardiac contractility also increases during isoproterenol administration and these changes are closely correlated with changes in heart rate (de Mey, et al., 1992; De Mey, Erb, Schroeter, & Belz, 1996). An additional limitation that may be addressed by future studies relates to the absence of a measure of breathing change in the cross correlation, since participants were instructed to rate respiratory as well as cardiac sensations. However, we feel that utilizing the heart rate reflects an acceptable approach for several reasons. Firstly, isoproterenol induced respiratory changes occur concomitantly with cardiac changes. Secondly, the heart rate is the most readily observable and commonly utilized measure of the response to isoproterenol in both clinical and research applications. Finally, in the current study reliable cross correlations were measured using the heart rate alone.

Another important consideration for the current study is the fact that all participants underwent two isoproterenol sensitivity tests prior to the interoceptive rating condition. Thus, each participant was familiar with the particular interoceptive sensations elicited by isoproterenol prior to the measurement of interoceptive awareness. Consequently, the interoceptive ratings could in principle have been biased by a learning effect (in the same vein, it is equally possible that the lack of increases in mental anxiety and distress could have been due to emotional habituation to the subjective experience of isoproterenol).

However, this intended feature of the design may have also improved the reliability of each participant's rating, by reducing the contribution of noise in the ratings related to novelty effects. A separate potentially confounding outcome of this design relates to habituation in the bodily response to isoproterenol. We found little evidence for this possibility, as an analysis of the CD25 values for all three rounds of isoproterenol failed to reveal any differences in sensitivity as exposure to isoproterenol increased. This absence of habituation bodes well for future research studies implementing isoproterenol, as it suggests that repeated administration of the same doses within the same participant will result in similar bodily responses.

A final consideration relates to the use of saline infusions in the current study. A minority of participants reported increases in heartbeat sensations during saline infusions, as indexed by the dial ratings and the overlap map of heartbeat sensations. This outcome is not surprising given the well known existence of the placebo response, and it is interesting to note that the frequency of placebo responses in the current study (30%) is entirely consistent with the rates of placebo responding in the literature (Olshansky, 2007). A more difficult question to answer may be why some individuals perceived interoceptive sensations during saline infusions. One potential explanation may be found from the literature on heartbeat detection. Since a minority of individuals are good heartbeat detectors at rest, it is likely that several individuals in the current study would also be classified as good detectors if tested on a heartbeat detection paradigm. It seems possible that during several saline infusions this type of individual might have been rating spontaneous cardiac changes occurring during the infusion interval as well as cardiorespiratory changes induced by the manipulation itself, such as cardiac accelerations and decelerations related to infusion administration (Vila et al, 2007). This question could be addressed in future studies.

The contribution of bodily signals to the experience of emotion has remained a fundamental and unresolved issue in emotion research, primarily due to the lack of suitable methods for manipulating the state of the body. The current findings indicate that bolus isoproterenol infusions provide a reliable method for manipulating and assessing interoceptive awareness. This method reliably demonstrated increases in interoceptive awareness in the majority of participants, thereby overcoming a major limitation of heartbeat perception tasks. The versatility of this approach in inducing brief, rapid and reversible changes in arousal suggests that it may help in providing new understandings of how conscious and subconscious feedback from the body influences the experience of emotion, how these experiences are mediated within the central nervous system, and how they might guide cognition and behavior.

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Fig 1. Mean heart rate response and retrospective ratings of interoceptive sensations as a function of bolus infusions of isoproterenol. (A) Mean heart rate response. (B) Retrospective ratings of the intensity of heartbeat sensations (scale: 0 = 'normal' to 10 = 'most ever'). (C) Post infusion wagering on the presence of heartbeat change during each infusion. (E) Retrospective ratings of the intensity of breathing sensations (scale: 0 = 'normal' to 10 = 'most ever'). (F) Post infusion wagering on the presence of breathing change during each infusion. (D) Affective measures (scale: 0 = 'normal' to 10 = 'most ever'). Error bars = SE.

Fig 2.

Time course of heart rate and online interoceptive dial ratings as a function of bolus infusions of isoproterenol. (A) Mean time course of heart rate at each dose (thick black line) and associated normalized dial rating (thick dashed gray line) for all participants. Thin lines indicate SE. (B) Cross correlation between objective heart rate increases induced by isoproterenol and subjective online ratings of the intensity of heartbeat and breathing sensations at zero lag. (C) Maximum cross correlation. (D) Absolute lag times at maximum cross correlation.

Fig 3.

(A) Percentage of participants rating increases in interoceptive sensations at each dose. A participant was considered to have endorsed an increase in sensation above baseline if they turned the dial above zero at any point during the 90 second infusion period. The numbers at the top of the bars denote the number of participants endorsing increased sensation at each dose. (B) Correlation between the peak dial rating of the intensity of interoceptive sensations and the peak heart rate change during the highest dose (2.0 mcg) for each participant. Dial rating scale: 0 = 'normal' to 10 = 'most ever'.

Fig 4. Locations of perceived heartbeat sensations following isoproterenol and saline infusions. All 15 participants' traced outlines of heartbeat sensations for each dose are overlapped, with hotter colors corresponding to a greater number of overlaps across participants.

Table 1

Demographic data for all participants. Chronotropic Dose 25 (CD25): dose of isoproterenol that would be required to increase the heart rate by 25 beats per minute. Means $\pm$ standard deviation.

Group demographics	
Sex	10 Men, 5 Women
Age (years)	37.0 $\pm$ 12.8 (range: 19-60)
Body Mass Index	25.2 $\pm$ 3.4 (range: 19.8-31.5)
Race	13 Caucasian American, 1 Asian American, 1 African American
Education (years)	15.7 $\pm$ 2.1
Beck Anxiety Inventory score	3.9 $\pm$ 4.0
Beck Depression Inventory score	3.9 $\pm$ 5.3
Chronotropic Dose 25 (micrograms)	
Isoproterenol sensitivity test	1 4.88 $\pm$ 2.65
Isoproterenol sensitivity test	2 6.99 $\pm$ 6.15
Interceptive rating trial	5.37 $\pm$ 3.95