Interoceptive cardiac signals selectively enhance fear memories

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CARDIAC SIGNALS ENHANCE FEAR MEMORIES

Abstract

Fear is coupled to states of physiological arousal. We tested how learning and memory of threat, i.e. conditioned fear, is influenced by interoceptive signals. Forty healthy individuals were exposed to two threat (CS+, paired with electrocutaneous shocks) and two safety (CS-) stimuli, specifically time-locked to either cardiac ventricular systole (when arterial baroreceptors signal cardiovascular arousal to brainstem), or diastole (when these afferent signals are quiescent). Threat learning was indexed objectively using skin conductance responses (SCRs). During acquisition of threat contingencies, cardiac effects dominated: Stimuli (both CS+ and CS-) presented at systole evoked greater SCR responses, relative to stimuli (both CS+ and CS-) presented at diastole. This difference was amplified in more anxious individuals. Learning of conditioned fear was established by the end of the acquisition phase, which was followed by an extinction phase when unpaired CSs were presented at either the same or switched cardiac contingencies. One day later, electrocutaneous shocks triggered the reinstatement of fear responses. Subsequent presentation of stimuli previously encoded at systole evoked higher SCRs. Moreover, only those participants for whom stimuli had the same cardiac-contingency over both acquisition and extinction phases retained conditioned fear memory (i.e. CS + > CS-). Our findings reveal two important cardiac afferent effects on threat learning and memory: 1) Cardiac signals bias processing towards threat. 2) Cardiac signals are a context for fear memory; altering this context can disrupt the memory. These observations suggest how threat reactivity may be reinforced and maintained by both acute and enduring states of cardiac arousal.

Psychological processes interact dynamically with bodily physiology (Critchley et al., 2013). Cognitions and emotions trigger patterned autonomic changes (Kreibig, 2010) across bodily systems, reflected in skin conductance responses (SCRs), heart rate, blood pressure and breathing. Conversely, perceptions, thoughts and feelings are sensitive to afferent signals concerning the state of body and the accuracy with which such information is detected and represented (James, 1884; Wiens et al., 2000), a process known as interoception. Memory processing and decision-making reflect this embodiment of cognition (Damasio et al., 1991; Kandasamy et al., 2016) in which feedback of internal states of bodily arousal may facilitate attentional deployment, memory processing (Bradley et al., 1992; McGaugh, 2004; O'Connell et al., 2008), and subjective confidence in judgements (Allen et al., 2016).

We have previously shown how information about cardiovascular arousal, originating in phasic interoceptive signals from the heart, may selectively enhance the processing of fear and threat signals, including facial expressions of fear (Garfinkel et al., 2014b) and the potential presence of a gun (Azevedo et al., 2017). The same signals also interfere with processing of other forms of (non-emotional) perceptual information (Edwards et al., 2009; Schulz et al., 2009). Together, these observations suggest a target mechanism to manipulate the perceptual and cognitive processes underlying abnormal emotional experience, for example phobic anxiety (Watson et al., 2019). The present study set out to understand these mechanisms in greater depth: Here, using an experimental fear-conditioning paradigm, we examined how learning and extinction of threat are influenced by afferent signals concerning cardiovascular arousal.

Interaction between physiological and psychological processes can be revealed through experimental manipulation of bodily state, e.g. cortisol administration will influence memory, with implications for understanding memory effects of endogenous cortisol release

during stress (e.g. Abercrombie et al., 2006; Okuda et al., 2004). One can also examine how natural fluctuations in physiological feedback impact specific mental processes: In the present study, we exploited cardiac signals. The beating of the heart triggers the phasic firing of arterial baroreceptors. These baroreceptors, located in the aortic arch and carotid sinus, are pressure-sensitive; they fire as the vessel walls are stretched by the ejection of blood from the heart at ventricular systole. In contrast, baroreceptor firing is quiescent between heartbeats, at diastole. Thus, afferent visceral signals occurring at ventricular systole inform the brain about state of cardiovascular arousal by encoding the timing and strength of heartbeats. Experimentally, the impact of this interoceptive channel can be tested by comparing responses to brief stimuli timed-locked to systolic and diastolic phases of the cardiac cycle (Garfinkel and Critchley, 2016). This methodology enables the psychological and behavioural impact of cardiovascular arousal signals to be studied selectively, without additional confounds of a generalised physiological arousal state (Garfinkel and Critchley, 2016).

There is well-documented evidence for an inhibitory influence of ventricular systole on stimulus processing. Systole, relative to diastole, attenuates pain responses including nociceptive flexion reflex to painful stimuli (McIntyre et al., 2006; McIntyre et al., 2008) and the subjective experience of pain (Dworkin et al., 1994; Edwards et al., 2002). Systole also attenuates startle responses to acoustic stimuli (Schulz et al., 2009), and impairs the encoding of words into memory (Garfinkel et al., 2013). This general inhibitory effect of cardiac systole on stimulus processing putatively reflects the primacy and attentional salience of internal (interoceptive) bodily sensations (Lacey and Lacey, 1978). However, this general model is now refuted by the demonstration of opposite, facilitatory effects of cardiac systole on the processing of specific types of emotional stimuli. In particular, systole enhances the

processing of threat stimuli and fear signals (Azevedo et al., 2017; Garfinkel and Critchley, 2016). For example, presentation of emotional face stimuli at cardiac systole amplifies the perceived intensity of facial expressions of fear. Similarly, when presented at the cusp of conscious awareness, systole selectively increases the likelihood of detecting of such fear signals (Garfinkel et al., 2014b). Within the brain, systolic timing enhances the reactivity of the amygdala to fear faces (Garfinkel et al., 2014b) and to electrocutaneous shocks (Gray et al., 2009). Thus, this specific cardiovascular afferent channel specifically facilitates the processing of transient fear and threat stimuli while other types of processing are dampened. However, it has not previously been documented whether this effect extends to fear/threat learning and memory. Here we integrate a fear-conditioning paradigm with the timing of cardiac phasic to test for predicted effects of interoceptive signalling of cardiovascular arousal on the learning and retention of fear memories.

This study has clinical relevance: In humans, anxiety is characterized by exaggerated and sustained threat reactivity. Anxious individuals often manifest altered interoception, including heightened subjective (self-reported 'sensibility '(Ehlers and Breuer, 1992; Naring and Vanderstaak, 1995)) and objective (heartbeat detection task accuracy (Dunn et al., 2010; Pollatos et al., 2007)) sensitivity to interoceptive sensations, yet anxiety symptoms are further predicted by mismatch between subjective and objective (performance accuracy) measures of interoception (Garfinkel et al., 2016; Paulus and Stein, 2006). In anxiety, these interoceptive differences mostly relate to the cardiovascular domain, further motivating our interest in how threat learning is influenced by afferent cardiac signalling. We therefore modified a conventional conditioned-fear learning and memory (acquisition, extinction, reinstatement) paradigm to incorporate a cardiac timing component. We included two CS+ (neutral shapes, paired with shock), one presented at diastole and one at systole, and two CS-s (neutral shapes,

never associated with an aversive stimulus), again one at systole and one at diastole (Achaibou, 2011). This manipulation created a cardiac 'context' for each CS and allowed us to test for the predicted enhancement of threat processing at systole during baroreceptor firing. The approach also permitted us to test for a contextual / congruency effect, similar to effects of environment on memory (Godden and Baddeley, 1975), in which interoceptive context can shape learning and retention, as observed for specific mood (Lewis and Critchley, 2003) or drug states (Duka et al., 2001). Thus, we hypothesized that brief cardiac state (systole or diastole) will facilitate memory when congruency is kept consistent during learning and consolidation. We explicitly tested for a specific influence of cardiac context by switching cardiac contingency: During extinction, half the participants processed each CS+ and CS- with the same cardiac contingency that they had experienced during acquisition of conditioned fear. For the other half of the participants, stimuli previously presented at diastole during acquisition were now presented at systole and vice versa. We measured objective and subjective indices of threat learning and fear memory. Objectively, we used stimulus-evoked skin conductance responses (SCRs, sympathetic electrodermal response) to index psychophysiological arousal to perceived threat. Unlike most other axes of sympathetic innervation, the SCR is cholinergically-mediated (i.e. not sensitive to changes in circulating adrenaline), is unopposed by parasympathetic innervation, and has no intrinsic afferent feedback. Moreover, unlike muscle sympathetic nerve activity, SCR is unaffected by baroreceptor activation / cardiac timing (Donadio et al., 2002). Subjectively, we used shock expectancy as an index of explicit knowledge of CS+ and CS- contingency, confirming acquisition of conditioned fear. Pleasantness ratings of the stimuli were also recorded as an indirect subject measure of their affective valence.

Taken together, three specific predictions were made: (1) Fear processing will be enhanced at systole; (2) this enhancement will be exaggerated in more anxious individuals, and; (3) switching cardiac contingencies during extinction will disrupt memory for the conditioned response, manifesting as a diminished differentiation of CS+ and CS- (Day 2).

Methods Participants

Power analyses based on prior research pertaining to cardiac effects demonstrate an average effect size of 0.52 (Azevedo et al., 2017; Garfinkel et al., 2014b), indicating a sample size of 25 is required to achieve 80% power. The 'return of fear', (e.g. elicited by a reinstatement procedure, following successful extinction) is well established (e.g. Hermans et al., 2005; Norrholm et al., 2006; Schiller et al., 2008). No previous study has utilised cardiac timing as an internal context, but it is well documented that the effects of fear reinstatement / recovery are sensitive to external context, as demonstrated in both animal and human experiments (e.g. LaBar and Phelps, 2005; Rescorla and Heth, 1975). Reinstatement effects for fear memory (using conditioning procedures) that are sensitive to context display effect sizes differing dependent on design and population. Previous work using fear conditioning procedures has detailed an average effect size of 0.82 (averaged across studies, LaBar and Phelps, 2005) for context-contingent reinstatement in humans, suggesting a sample of 19 would be sensitive to display this effect at 80% power. Together, a total sample size of 40 was chosen to cover the cardiac-timing effects and further accommodate the contextual manipulation.

Forty healthy volunteers (20 females), recruited through poster and online advertisements at the University of Sussex and in the local area (Brighton and Hove), participated in this study, receiving re-imbursement for their time. The age of participants ranged from 18 to 37 years, with a mean age of 23.4 years (SD= 4.8). Each participant completed the Spielberger State-Trait Anxiety Inventory to quantify individual differences in trait anxiety (Spielberger et al., 1983). Thirty-nine participants were right handed. The Research Governance and Ethics Committee of Brighton and Sussex Medical School,

University of Sussex (BSMS RGEC) approved the experiment. All research was conducted in accordance with relevant governance guidelines and regulations with participants providing informed consent prior to participating.

Fear conditioning paradigm and procedure

The experiment consisted of three different phases over two sequential days: Day 1 -- acquisition of conditioned fear (*conditioning phase*) and then an *extinction phase*, Day 2 -- *reinstatement phase*, including test of reinstatement.

All components of the experimental procedure delivered using a computer-driven task, written in-house (Matlab, Natick, MA), in which stimulus presentation (including triggering of electrocutaneous shocks) was time-locked to the participant's electrocardiogram (ECG).

On Day 1, during the *conditioning phase*, four neutral stimuli (black abstract shapes on grey background) were used as conditioned stimuli (CS) (Hogarth et al., 2008). Two of the CS were followed by an aversive event (unconditioned stimulus; US) in the form of an electrocutaneous shock for 70.6% of the trials (partial reinforcement paradigm). This CS-US pairing conditioned two CS+ stimuli through association. The remaining two stimuli were unpaired, i.e. never followed by shock, thus forming CS-. We incorporated a cardiac timing manipulation by presenting one CS+ was time-locked to cardiac systole and the other to cardiac diastole. Similarly, one CS- was also time-locked to systole, while the other was presented at diastole. The initial learning phase lasted for 112 trials, with each of the four CS types (CS+systole, CS+diastole, CS-systole, CS-diastole) presented 28 times, fully intermixed throughout the task. Stimuli were present onscreen for screen for 100ms,

sufficient for perceptual discrimination and encoding of shape identity. In paired trials, the US immediately followed CS+ offset. Inter-stimulus interval was a minimum of 8 seconds, typically longer and influenced by resting heart rate for contingent stimulus presentations (Figure 1).

Also on Day 1, following acquisition, newly learned fear was extinguished in a *fear extinction* phase, by repeated presentation of all four CSs in the absence of the US (shock). As in the initial conditioning phase, there were 28 presentations for each CS, resulting in 112 presentations in total. The *extinction phase* followed a between-participant design, in which approximately half of the participants (N=19; randomly assigned) experienced extinction with the same stimulus-heart contingencies (CS+ and CS- were presented at the timing period within the cardiac cycle as during the conditioning phase). For the remaining participants (N=21), stimulus-heart contingencies were switched during extinction (stimuli previously presented at systole were presented at diastole and vice versa).

Finally, in a *fear reinstatement* phase on Day 2, the (US-triggered) recovery of the fear memory was tested, providing insight into whether cardiac signals influence the return of previously learned and extinguished fear memories. Participants experienced the US (4 electrocutaneous shocks) at the beginning of the task. These shocks were not time-locked to the cardiac cycle. To test reinstatement, over 60 intermixed trials, each CS was presented 15 times without the US. In this final phase, none of the CSs was specifically time-locked to systole or diastole (Figure 1).

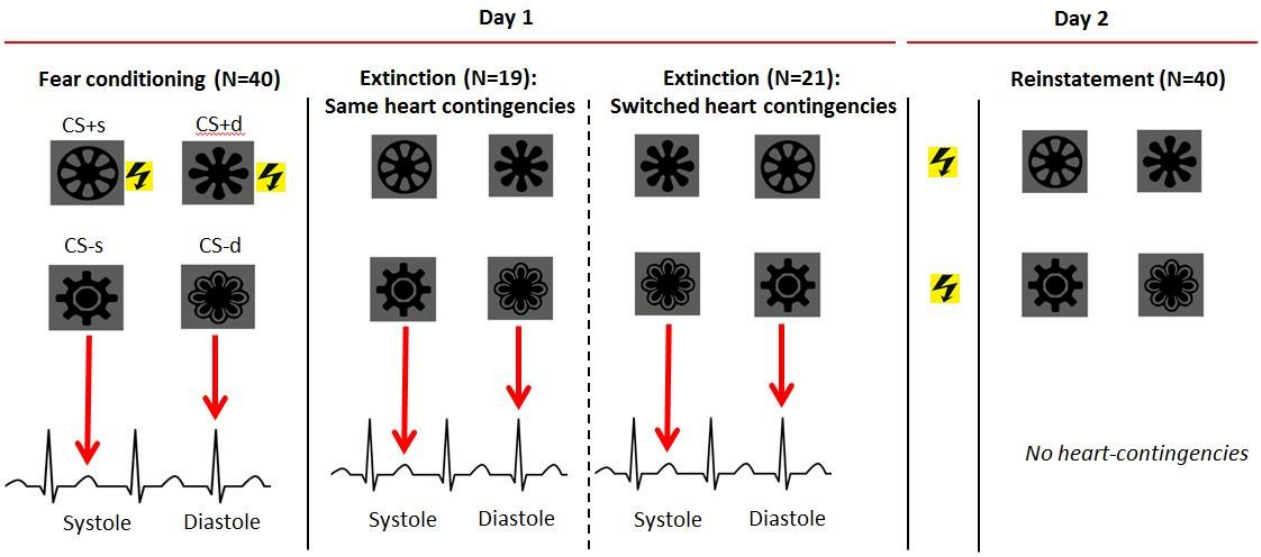

Figure 1. *To establish conditioned fear learning, conditioned stimuli (CS) were paired with a shock (CS+) or no shock (CS-) and time-locked to either systole (CS+s, SC-s) or diastole (CS+d, CS-d). During extinction, when no shocks were presented, half of participants (N=19) were shown the CSs with the same heart contingencies, while the other half of participants (N=21) had these contingencies switched, i.e. those stimuli originally viewed at systole were now seen at diastole, and those originally viewed at diastole were now seen at systole. On day 2, participants were exposed to random shocks (to reinstate the fear memories) and were then shown all conditioned stimuli. During this reinstatement phase, CSs were not time-locked to the cardiac cycle.*

For all experimental phases, the participant sat in a quiet room in front of an 18-inch monitor, with arms rested on a cushion. She/he followed instructions to look at the presented pictures and move as little as possible. After each of the three phases, as a subjective measure of conditioning, the participant rated for each picture her/his expectancy of a shock using a continuous Visual Analogue Scale (VAS) between 'Never' and 'Every time it is presented'. Before and after each experimental phase, the participant rated the perceived pleasantness of

each stimulus also using a continuous VAS (‘not at all pleasant’ to ‘extremely pleasant’). In both cases, only the ends of the VAS were labelled.

Delivery of US and electrocutaneous stimulation thresholds

Brief electrocutaneous shocks were delivered using a Constant Current Stimulator (Model DS7A®, Digitimer Ltd, Hertfordshire, England; compliant for clinical use within the European Community). Current strength could be varied between 0 and 100 mA. The skin on the left forearm of the participant was cleaned (Cutisoft®, BSN MedicalGMBH, Hamburg, Germany) before affixing two Ag/AgCl electrodes covered in conductive paste (Ten20®, D.O. Weaver and Company, Aurora, US) with surgical tape. These were connected to the stimulator. Each participant’s individual stimulation threshold was then determined by taking the mean value of current strengths that were experienced as highly annoying but not painful (Garfinkel et al., 2014a; Orr et al., 2000). To find this level, current strength was increased in small steps starting from 0 until the participant experienced any signs of pain (as ascertained via yes/no self-report, P1), then it was increased once more before decreasing incrementally until the participant no longer reported pain (P2). After another step down, this step-wise procedure was repeated twice to find points P3 –P6 before the mean of all six was taken as the threshold (Ascending-Descending Method of Limits; Dixon, 1965). During the experimental procedure, all electrocutaneous shocks were delivered as continuous 50ms pulses for a total duration of 200ms.

Electrodermal activity (Skin Conductance Responses; SCRs)

Two Ag/AgCl electrodes were attached to the fingertips of the participant’s index and middle fingers of the right hand and attached to a Skin Conductance Module (CED 2502®, Cambridge Electronic Design Ltd, Cambridge, England). The sensor electrodes were attached

using surgical tape to the skin and conductive paste was applied in between. The electrodermal signal was relayed via an analogue-to-digital converter (CED-Power 1401®, Cambridge Electronic Design Ltd) to the recording software, Spike (Version 2.6, Cambridge Electronic Design Ltd). Responses were sampled at 100 Hz (Lim et al., 1997). Electrodermal activity was recorded during all phases of the fear conditioning, extinction and reinstatement procedure.

Electrocardiogram (ECG)

Each participant underwent electrocardiography (single channel ECG) for the duration of the experiment. The skin on the left and right upper chest and the left lower back was cleaned (Cutisoft® wipes) and three Ag/AgCl monitoring electrodes with foam tape and sticky gel (3M HealthCare, Neuss, Germany) were attached. Electrodes were connected to an ECG Electrode Adaptor (CED 1902-11, Cambridge Electronic Design Ltd). The signal was amplified (CED 1902 Quad System, Cambridge Electronic Design Ltd) and sent through the analogue-to digital converter (CED-Power 1401) to a second channel in Spike. A real-time script running on the CED-power1401 unit, identifying the QRS complex with millisecond temporal accuracy, controlled the ECG-triggered timing of stimulus presentation. Stimulus presentation was time-locked to either the R-wave (end of cardiac diastole) or delayed 300 ms from the R-wave peak, coincident with systole (Garfinkel et al., 2014b; Gray et al., 2009) when baroreceptor impulses are processed centrally (Edwards et al., 2007; Edwards et al., 2009; Gray et al., 2009).

Anxiety

Trait anxiety symptomatology was ascertained using the trait portion of the Spielberger Stait-Trait Anxiety Inventory (STAI) (Spielberger et al., 1983). This consists of 20 self-report items each with a 4-point Likert scale (1. Almost never, 2. Sometimes, 3. Often, 4. Almost always), generating a final score that varies from 20-80. Questions incorporate both anxiety present e.g. "*I feel nervous and restless"* and anxiety absent e.g. "*I am content"* questions, with the latter reversed scored.

Interoceptive accuracy

The *heartbeat discrimination test* (Garfinkel et al., 2015; Katkin et al., 1983) was used to determine individual differences in interoceptive accuracy. Individual heartbeats were monitored using a finger pulse oximeter (Nonin 8600 with a 'soft' sensory fitting to reduce exteroceptive feedback). Tone presentation (440 Hz with 100ms duration) was either timelocked to the participants heartbeat (generated at the beginning of the rising edge of the pulse pressure wave) or presented asynchronously (delayed by 300ms). Adjusting ~250ms for the pulse transit time, this resulted in tones being presented at around 250ms or 550ms after R wave, for the synchronous and asynchronous conditions respectively. Participants underwent 20 trials, and each trial consisted of the presentation of ten tones, presented either simultaneously or delayed with the participants own heartbeat. At the end of each trial, participants judged whether the tones were synchronous with their own heartbeats (yes / no) and accuracy scores were calculated (probability of being correct).

Data processing and analysis

Skin Conductance Responses (SCRs)

All data were smoothed using a Gaussian smoothing function in Matlab. For every stimulus, the average SCR level for the 2 sec time window prior to the onset was calculated as a baseline. The average amplitude in an 8 second post-stimulus time window was taken as the SCR response (in accordance with the mean typical latency to peak 4s, recovery time 4-20s seconds) (Dawson et al., 2017). Change in SCR was defined as the difference between the average amplitude response and the baseline. In order to capture fully individual differences, and the potential inhibitory effect of the heart, differences between baseline and post-stimulus amplitude were computed for all trials for all participants, even in the absence of a response. These were then averaged across all trials (72 trials for fear conditioning, 112 trials for extinction, 60 trials for reinstatement), separately for each stimulus (CS+systole, CS+diastole, CS-styole, CS-diastole). For fear conditioning, only unpaired CS+ stimuli were included in analyses to avoid the contamination of the shock into SCR responses to the stimulus (i.e. 72 out of the 112 trials were analysed). Once the data was fully processed (all participants, for all stimuli), extreme outliers for SCR were identified (using box-and-whisker plots, defined as above Q3 + 3 IQR). These participants were removed from data analyses as they radically skewed the data (one participant was removed from fear conditioning and extinction SCR analyses, and two were removed from reinstatement SCR analyses). Data were then checked for normality; data from both fear conditioning and reinstatement were normally distributed, verified using the Shapiro Wilk test ($W(39)=0.96$, $p=0.14$; $W(38)=0.95$, $p=0.114$, respectively). However, extinction data did not meet criteria for normal distribution $W(38)=0.904$, $p=0.003$) and underwent a square root transformation (Braithwaite and Watson, 2015), resulting in normalization ($W(39)=0.976$, $p=0.566$). All descriptive statistics (mean / SEM) are presented on baseline corrected raw data.

Data from each phase (conditioning, extinction, reinstatement) were analysed in their entirety to ensure all data-points were included. In addition, due to the temporal dynamics of conditioned learning, and consistent with previously published approaches, the end of the conditioning phase (last third of trials (40)) were separately analysed to demonstrate that conditioned fear learning had been established (Garfinkel et al., 2014a; Milad et al., 2005; Milad et al., 2007).

Analyses

A 2 x 2 Repeated Measures Analysis of Variance (ANOVA) with CS type (CS-, CS+), cardiac cycle (systole, diastole) was applied throughout SCR analyses. To assess conditioned learning, the dependent variable was SCR response minus baseline for each different type of stimulus (unpaired (i.e. no US) CS+systole, unpaired CS+ diastole, CSsystole, CS-diastole). Only the unpaired CS+ stimuli were included to avoid contamination of the SCR by the shock. Fear conditioning analyses also included interoceptive accuracy (heartbeat discrimination score) as a co-variate to control for effects of the interoceptive channel on learning (Garfinkel et al., 2015; Watson et al., 2019). For analyses pertaining to anxiety, the impact of individual differences in trait anxiety was tested using trait anxiety (STAIT score) as a continuous variable entered as a covariate. The range of scores on the STAI was 29-65 (mean=43.6; standard deviation=9.2).

Fear and conditioned fear learning were assessed using both a physiological signal (SCR to conditioned stimuli) and subjective report. Shock expectancy ratings and pleasantness ratings served as a subjective report dependent variables towards each of the

CSs following the three experimental phases (fear acquisition, extinction and reinstatement). Subjective pleasantness ratings to conditioned stimuli were obtained as an indirect test of conditioned learning, wherein increased aversiveness acquired during fear learning was predicted to manifest as decreased pleasantness relative to baseline. To mitigate for potentially confounding baseline differences, all ratings acquired at the end of each experimental phase were adjusted relative to baseline. Mirroring SCR analyses, repeated measures ANOVAs 2 x CS type (CS-, CS+), x 2 cardiac cycle (systole, diastole) were run with subjective ratings (either shock expectancy or pleasantness) as the dependent variable. Again paralleling SCR analyses, the impact of individual differences in trait anxiety on subjective ratings was tested using trait anxiety (STAIT score) as a continuous variable entered as a covariate, specific to analyses pertaining to anxiety. For extinction and reinstatement analyses of both SCR and subjective report, heart-timing contingency group (i.e. same verses switched) was added into the repeated measures ANOVA as a betweensubject factor. The average anxiety level between the same (mean = 41.94, SD =8.51) and different (mean=44.90, SD=9.63) contingency groups did not significantly differ [t(38)=-
1.02, p=0.32].

Results

Effect of cardiac timing on acquisition of conditioned fear

Over the entire conditioning phase, the effect of cardiac cycle dominated SCRs, manifesting as a main effect of cardiac cycle [$F(1, 36)=8.45$, $p=0.006$]. SCRs were significantly elevated at systole [$M=0.028$, $SEM=0.007$] relative to diastole [$M=0.009$, $SEM=0.005$] (Figure 2). In contrast, across the entire conditioning phase, there was no main effect of CS, where on average, SCRs were not significantly elevated to CS+ [$M=0.019$, $SEM=0.006$] relative to CS- [$M=0.017$, $SEM=0.005$] [$F(1, 36)=1.10$, $p=0.30$]. These observations are consistent with the prediction that cardiac signals (at systole) enhance fear responses, here generalising threat processing and apparently obstructing differential associative learning of threat (CS+) and safety (CS-). The cardiac cycle effect interacted with stimulus type [$F(1, 36)=6.61$, $p=0.01$]; where the effects were observed to a greater extent to CS- relative to CS+ stimuli. Specifically, SCR was significantly elevated for CS- at systole [$M=0.024$, $SEM=0.006$] compared to CS- at diastole [$M=0.010$, $SEM=0.005$] [$t(38)=-2.49$, $p=0.02$] while the SCR elevation for CS+ at systole [$M=0.031$, $SEM=0.009$] relative to CS+ at diastole [$M=0.007$, $SEM=0.007$] did not meet threshold significance [$t(38)=-1.83$, $p=0.08$] (Figure 2). Individual differences in interoceptive accuracy interacted with the cardiac cycle effect [$F(1, 36)=5.35$, $p=0.03$], whereby those individuals with high interoceptive accuracy had a stronger enhancement of SCR at cardiac systole compared to diastole, relative to individuals with poor interoceptive accuracy. There was no overall effect of interoceptive accuracy on SCR [$F(1, 36)=0.26$, $p=0.61$].

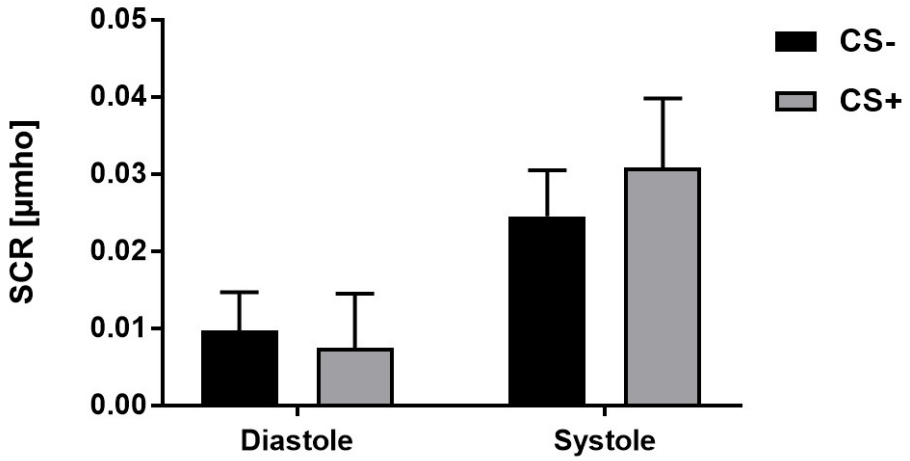

Figure 2. Effects of cardiac signals on responses to stimuli during fear conditioning. *Fear conditioned learning of the CS+ / CS- contingencies was disrupted by the cardiac manipulation, as participants displayed heightened SCR to both CS+systole and CS-systole, and reduced SCR to both CS+diastole and CS-diastole. Bars represent Mean+/- SEM.*

Late fear conditioning and the establishment of conditioned fear

Toward the end of the experimental session (last third of trials), participants learnt the contingent associations between stimuli and electrocutaneous shock, CS+ (both at systole and diastole) evoked greater SCRs [*M*=0.041, *SEM*=0.009] compared to CS- stimuli (presented at both systole and diastole) [*M*=0.032, *SEM*=0.009]. This was apparent as a main effect of stimulus type [F (1, 36)=4.47, *p*=0.04], in the absence of a main effect of cardiac cycle [F (1, 36)=3.63, *p*=0.07] and no CS by cardiac timing interaction [F (1, 36)=0.09, *p*=0.78]. Interoceptive accuracy no longer interacted with cardiac cycle F(1, 36)=2.73, *p*=0.11].

Effect of trait anxiety on fear responses.

We tested the second prediction that effects of cardiac systole on threat processing is enhanced in people who score high in trait anxiety. Again, using SCRs as an objective index of threat processing, we tested whether individual differences in trait anxiety (entered as a covariate) determined the cardiac effect during the entire *conditioning phase*. Overall SCR reactivity as a function of trait anxiety symptoms was elevated during fear conditioning [F (1, 35)=5.63, p=0.02]). In addition, differential SCRs to stimuli (both CS+ and CS-) presented at systole (relative to diastole) were elevated for individuals with high trait anxiety. This was apparent as a significant anxiety by cardiac timing interaction [F (1, 35)=5.00, p=0.03] (Figure 3).

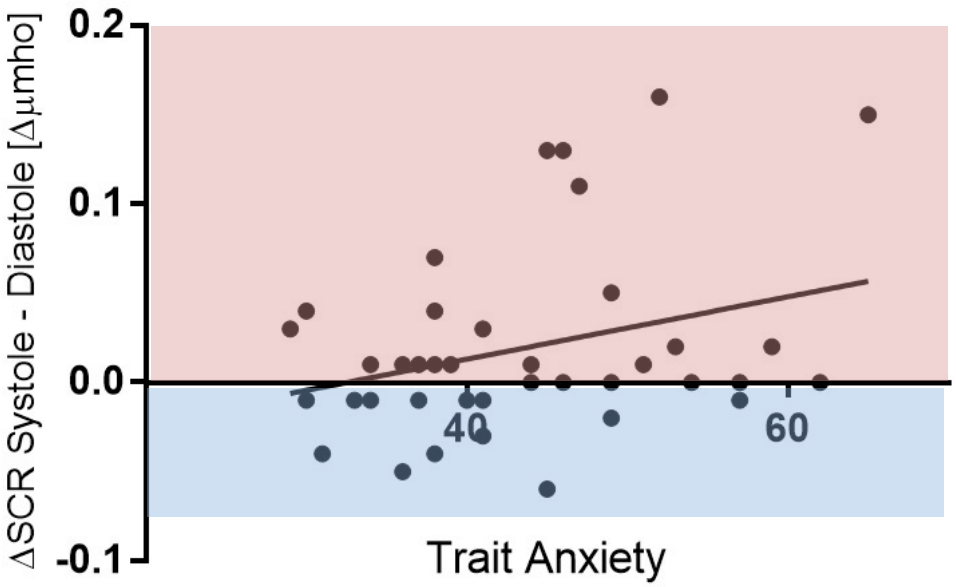

Figure 3. Anxiety is linked to cardiac effects on responses to stimuli*. The elevated response to conditioned stimuli at systole (averaged across CS+ and CS-) relative to those presented at diastole, was magnified in individuals with higher trait anxiety. Data points within the top (red) portion of the graph represent individuals who demonstrated a net elevation of SCR*

during cardiac systole, while blue represents individuals who, on average, displayed reduced SCRs at cardiac systole relative to diastole.

Subjective reports

Subjectively, fear conditioning was robustly established at the end of the *conditioning phase*, as demonstrated by participants' heightened expectation of shock to CS+ compared to CS- (main effect of conditioned stimulus type [$F(1, 38)=61.22$, $p<0.001$] for shock expectancy ratings). Shock expectancy at the end of the *conditioning phase* was also not modified by cardiac timing (i.e. no significant effect of cardiac timing on shock expectancy [$F(1, 38)=0.13$, $p=0.72$] and no cardiac timing- by-CS interaction [$F(1, 38)=1.62$, $p=0.21$], Figure 4A).

In addition to rating shock expectancy as a direct subjective measure of fear conditioning, participants also rated the pleasantness of the stimuli (an indirect measure of affective salience). This also supported the establishments of conditioned fear: At the end of the *conditioning phase,* CS+ stimuli were rated as less pleasant than CS- stimuli following the fear conditioning procedure [$F(1, 38) = 20.12$, $p<0.001$]. Additionally, rated pleasantness showed a significant cardiac timing by CS interaction [$F(1, 38) = 7.07$, $p=0.011$]. For stimuli presented at diastole, CS+ (threat stimuli) were perceived as much less pleasant than CS- (safe stimuli) [$t(38)=-4.71$, $p<0.001$]. However, for stimuli presented at systole, there was no significant difference in rated pleasantness of CS+ and CS- [$t(38)=1.56$, $p=0.13$] (Figure 4B). This suggests an enduring aversive effect of cardiac systole (i.e. baroreceptor afferent signals) on emotional responses to stimuli during fear learning.

Trait anxiety did not significantly modify subjective ratings of shock expectancy or pleasantness (main effect of anxiety group and all interactions with anxiety group p <0.2).

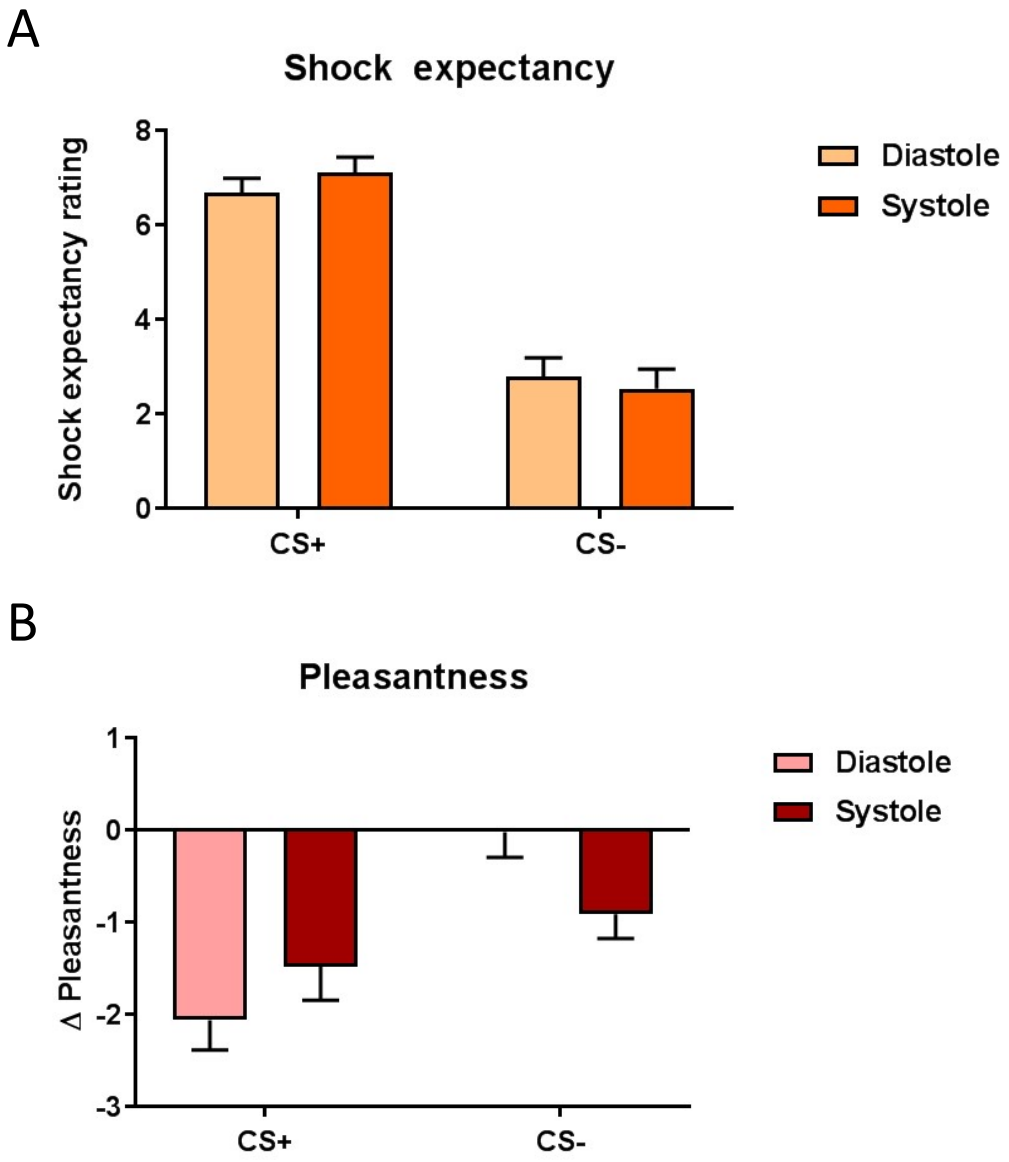

Figure 4. Expression of fear conditioning in subjective reports. ***A)*** *Ratings of shock expectancy were greater for CS+ relative to CS-, but were not affected by cardiac timing.* ***B)*** *Subjective ratings of pleasantness were decrease for all stimuli relative to baseline following the fear conditioning procedure, with the exception of the CS- presented at diastole.*

Fear extinction

The *extinction phase* followed the establishment of conditioned fear in the *conditioning phase* as described above. Presentation of unreinforced stimuli (CS+ without pairing with US) was associated with reduction of fear responses to the CS+. Averaging SCR responses across the entire extinction phase demonstrated that fear extinction was effective. There was no main effect of CS [F(1, 37)=0.39, p=0.54] [CS-(M=0.055, SEM=0.007), CS+(M=0.046, SEM=0.007)], no main effect of cardiac timing [F (1, 37)=0.66, p=0.42] [diastole (M=0.054, SEM=0.008), systole (M=0.048, SEM=0.007)] and no CS by cardiac timing interaction [F (1, 37)=1.68, p=0.20]. Moreover, the contingency group effect did not interact with cardiac timing [F (1, 37)=1.91, p=0.18] or CS [F (1, 37)=0.37, p=0.55]. Across the entire extinction phase, the SCR responses were not elevated with trait anxiety [F(1, 36)=0.18, p=0.68] and trait anxiety did not interact with cardiac cycle [F(1, 36)=1.81, p=0.19]. However, trait anxiety significantly interacted with CS, manifesting as enhanced SCR to the CS+ relative to CS- as a function of heightened anxiety symptomatology [F(1, 36)=19.57, p<0.001], an effect that occurred in the switched contingency group only [F(1, 19)=18.71, p<0.001] while not manifesting in the same contingency group [F(1, 19)=2.96, p=0.11]. Estimated marginal means were computed for the switched contingency group, to reveal elevated SCR at CS- [M=0.27 SEM=0.04] relative to CS+ [M=0.13 SEM=0.03] for anxiety -1SD, and the reverse pattern for anxiety +1SD, where SCR was lower for CS- [M=0.18 SEM =0.03] relative to

CS+ [M=0.23 SEM =0.03]. For the same cardiac contingency group, estimated marginal means were computed for CS- and CS+ at anxiety – 1SD [M=0.19 SEM =0.02 and 0.17

SEM=0.03, respectively], and for CS- and CS+ at anxiety +1SD [*M*=0.18 *SEM* =0.03 and *M*=0.21 *SEM* =0.03, respectively].

Reinstatement

On Day 2, two unpaired electrocutaneous shocks (US) were delivered to the participants; a conventional procedure for reinstatement of fear responses. Participants were then tested on their response to unpaired CSs. We observed no main effect of CS on fear responses (SCR) [F (1, 36)=2.40, *p*=0.13], showing that prior association with shock was *insufficient on its own* to evoke threat responses at restatement after extinction (CS- [*M*=0.37, SEM=0.008]; CS+ [*M*=0.053, *SEM*=0.010]). However, there was a significant main effect of heart-timing [F (1, 36)=5.89, *p*=0.02]: Stimuli (both CS+ and CS-) previously conditioned at systole [*M*=0.059, *SEM*=0.009] were associated with a greater SCR fear response at reinstatement relative to those conditioned at diastole [*M*=0.031, *SEM*=0.008] (Figure 5). There was no significant CS by cardiac timing interaction [F (1, 36)=0.13, *p*=0.72].

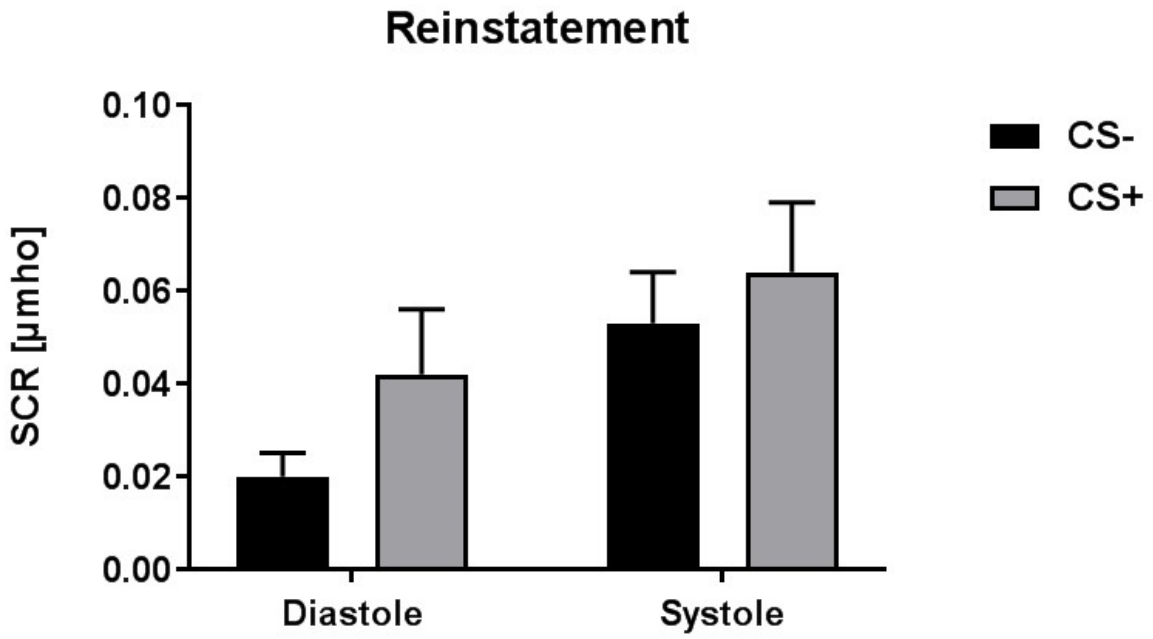

Figure 5. The facilitatory effect of the heart on fear memories. *Following reinstatement, SCR response was heightened to all stimuli previously conditioned at systole, irrespective of whether they were formally associated with shock.*

Reinstatement reveals contextual effects of cardiac signalling

A prior effect of conditioning was nevertheless manifest as a CS by contingency interaction [F (1, 36)=7.33, *p*=0.01]. Those individuals who underwent fear extinction within the same cardiac contingencies had elevated SCR to CS+ [*M*=0.063, *SEM*=0.015] relative to CS- [*M*=0.013, *SEM*=0.006]. This demonstrated a selective fear reinstatement effect [t (17)=3.09, *p*=0.007]. There were no differences in SCR responses to CS+ [*M*=0.045, *SEM*=0.012] and CS- [*M*=0.058, *SEM*=0.011] for those individuals who extinguished over a different heart contingency, [t (19)=0.81, *p*=0.43] (Figure 6).

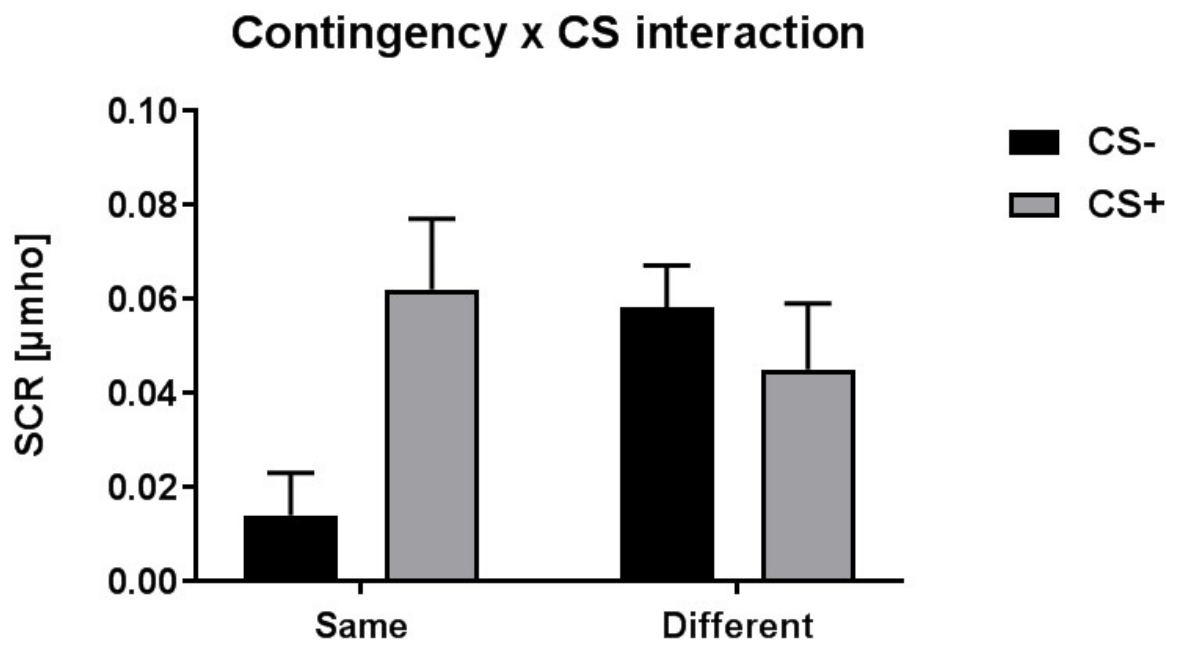

Figure 6. Heart as an internal context*. Following reinstatement, the SCR fear response was selectively elevated to the CS+ only in the group who were previously extinguished on using the same cardiac contingencies.*

On day 2, no effects of anxiety on SCR were significant during this *reinstatement phase.* There was no main effect of anxiety [$F(1, 35)=0.81$, $p=0.38$], no anxiety by CC interaction [$F(1, 35)=2.25$, $p=0.14$] and no anxiety by CS interaction [$F(1, 35)=0.02$, $p=0.89$].

Discussion

We used cardiac timing manipulations during fear conditioning and extinction to characterise the influence of afferent cardiovascular signals on fear learning and fear memory. There were three major findings: First, conditioned stimuli presented at cardiac

systole evoked greater threat responses. This effect was particularly apparent during conditioned learning where systole, and by extension arterial baroreceptor signalling, overshadowed differential learning of fear and safety cues, eliciting a threat response for stimuli presented at systole, irrespective of whether they were CS+ or CS-. By extension, diastole served as an intrinsic safety cue, reducing SCR response to both CS+ and CS-. The impact of cardiac timing on threat processing also extended to Day 2, when fear reinstatement reactivated threat responses, i.e. fear memory, to stimuli originally presented at systole. Second, we observed during initial learning (Day 1) that the amplification of fear responses at cardiac systole was exaggerated in individuals with high trait anxiety. Third, the memory of established threat (CS+ vs. CS-) was also sensitive to the short-term cardiovascular context (systole and diastole) and thus was disrupted when these contingencies were switched between *learning* and *extinction phases*. Together these data show that threat reactivity, fear learning, and its retention, are guided by phasic afferent signals from the cardiovascular system.

Our data show that threat reactivity is dependent on information relating to the state of cardiovascular arousal: Cardiac systole amplifies psychophysiological (SCR) responses toward real and potential threat. Indeed, this enhancing effect of systole on threat processing competed for dominance with conditioned fear acquisition, delaying the discriminatory learning of fear and safety (CS+ vs. CS-) while instead generalising fear responses (SCRs) to stimuli presented at systole, irrespective of whether they were reinforced by electrocutaneous shock. Thus, cardiac signals led to a heightened fear response to a ‘safe’ stimulus within this experimental setting. An enduring facilitatory effect of systole on fear memories was also evident on Day 2, when following a reinstatement procedure, there was a selective return of threat responses to stimuli that had been consistently associated with cardiac systole on Day

1. The mechanism through which these interoceptive signals contribute to threat processing originates in the activation of arterial baroreceptors by ejection of blood from the heart at cardiac systole, signaling to brain the timing and strength of each heartbeat. This afferent channel thus represents the body-to-brain mechanism through which cardiovascular arousal is communicated to the brain. While stimulus processing has a tendency to dampen or inhibit most types of processing (Lacey and Lacey, 1978), these findings are consistent with a growing literature that suggests one noticeable exception is fear processing, which has the capacity to be *facilitated* at cardiac systole (Garfinkel and Critchley, 2016), an effect mediated by selective systolic enhancement of amygdala activity (Garfinkel et al., 2014b). This is the first study to examine the impact of systolic signals on fear conditioning, thereby extending evidence for cardiac enhancement of fear processing. Fear faces are judged as more intense at systole relative to diastole (Garfinkel et al., 2014b), and fear faces and electric shocks administered at systole are both associated with enhanced activity within the amygdala, a key neural substrate for fear learning and threat response (Garfinkel et al., 2014b; Gray et al., 2009). Moreover, fear faces presented at the cusp of perceptual awareness (during an attentional blink paradigm), are more likely to breakthrough into consciousness when time-locked to systole (Garfinkel et al., 2014b). This latter observation highlights the selective impact of cardiac afferent signals on attentional capture by emotionally salient threat stimuli, a process that is amygdala-dependent (Anderson and Phelps, 2001). This facilitating effect of fear processing at systole is an exception to predominantly inhibitory effects of systole on other kinds of sensory processing (Lacey and Lacey, 1978). Pain responses are reduced at systole (Dworkin et al., 1994; Edwards et al., 2002; McIntyre et al., 2006; Rau and Elbert, 2001), eye-blink startle is attenuated (Schulz et al., 2009), blood pressure response to shocks is dampened (Donadio et al., 2002; Wallin, 2007) and muscle sympathetic activity to shocks is reduced (Gray et al., 2009) (see Garfinkel and Critchley,

2016 for a review). Our results also show that the augmentation of fear learning and fear memories at systole occurs at the cost of learning to differentiate CS+ from CS-. In humans, studies of fear conditioning demonstrate the capacity for individuals to acquire selective conditioned fear responses, demonstrable as increased SCR for events (CS+) previously associated with an aversive outcome (US, e.g. electrocutaneous shock) relative to a safe stimuli (CS) (LaBar et al., 1998; Milad et al., 2007; Phelps and LeDoux, 2005). Here we observed evidence for a partial overshadowing of fear responses at systole, to the detriment of differentiating between CS- and CS+.

In contrast to 'automatic' physiological expression of threat leaning (SCRs), we were unable to track over time whether conscious, cognitive learning of threat, indexed by shock expectancy ratings for CS+ versus CS- showed an initial dependence on cardiac timing. However, the participants reliably stated their explicit expectation of the electrocutaneous aversive shock (US) to CS+ (in contrast to CS-) stimuli at the end of the *conditioning phase*. Thus, while subjective report of shock expectancy showed higher-order learning and knowledge of threat, this effect was potentially dissociable from the bodily expression of fear learning which at least initially showed a dependence on cardiac phase. While this might suggest the interplay of parallel systems for fear learning (Kitamura et al., 2017; Rashid et al., 2016), subjective ratings of stimulus pleasantness (an indirect, yet also subjective measure of aversive learning) did however also demonstrate a cardiac effect. Following fear conditioning, CS- at systole was rated as less pleasant than CS- at diastole. Thus, the cardiac enhancement of fear response was observed in both early physiological measures (SCR) and in affective appraisal processes (pleasantness ratings) which can be dissociated from awareness of shock expectancy awareness (Jeffs and Duka, 2017). These findings provide a

partial demonstration of dissociation between autonomic and cognitive expressions of threat learning (Critchley et al., 2002).

Cardiac afferent signals enhance threat reactivity in people who are more anxious: During the conditioning phase, systole increased SCRs to stimuli in people who experienced most trait anxiety symptoms; i.e. both CS+ or a CS-, providing they were at systole, elicited a greater SCR in individuals with higher trait anxiety. This has potential clinical relevance to conditions characterised by exaggerated fear reactivity and recurrent fear memories notably Post Traumatic Stress Disorder (PTSD) (Rachman, 1989). Understanding how cardiac afferent signals underscore augmented fear learning could provide insight into the pathophysiology of fear-related conditions. Recognition and further characterisation of how cardiac afferent signals can heighten fear responses in high anxious individuals may be useful in the future management of anxiety disorders. Moreover, determining whether these effects are further exaggerated in individuals who meet formal diagnostic criteria for anxiety is also of interest.

Pre-clinical studies highlight how context can effect learning, memory and extinction of conditioned fear. The context is usually external, environmental and relatively stable. Nevertheless, in the present study we hypothesized that short-term interoceptive signals, i.e. the phase of cardiac cycle, would show similar context effects. One key prediction was that memory would be strengthened if the interoceptive content experienced during encoding persisted. In accordance with this prediction, we observed that, on Day 2, differential responses to CS+ and CS- were only present in individuals who had acquired and extinguished fear responses within the same cardiovascular context. Switching the cardiac timing associated with CS+ presentation, from systole at acquisition to diastole at extinction or vice versa, cardiac cycle disrupted established threat memories. Altered extinction learning

was also observed in the switched cardiac contingency group, emerging as a function of anxiety. Together these results support the notion that cardiovascular arousal state (here reduced to the phasic signals from baroreceptors at systole) provides an internal context, which if consistent, improves memory formation, consolidation and retention, fostering accurate subsequent recall. These findings build upon evidence from manipulations of external context (Godden and Baddeley, 1975), studies of mood congruent memory (Lewis and Critchley, 2003) and experimentally induced pharmacological alterations in internal state (Duka et al., 2001). Again, there are clinical implications: Individuals with PTSD are impaired in their capacity to utilize external context to guide appropriate memory expression (Garfinkel et al., 2014a). Cardiovascular arousal signals may dominate as an internal context and thus contribute to the pathophysiology of facilitated fear memories. Our findings show that the affective properties of a stimulus may be disrupted by fluctuations between high and low cardiovascular arousal. The baroreceptor afferent channel is implicated as a route through which physiological congruency between earlier stimulus processing and later recollection impacts memory strength.

The behavioural expression of fear conditioning can be displayed in the absence of peripheral autonomic responses (e.g. in individuals with pure autonomic failure (Critchley et al., 2002) or in animal studies of peripheral autonomic blockade (Do Monte et al., 2008)). Nevertheless, the afferent feedback of the cardiovascular responses to threat serves as a contextual influence on how fear learning occurs, and in the maintenance of fear memory. Correspondingly, brain systems (e.g. amygdala, insula and hippocampus) supporting fear learning are sensitive to signalling of physiological arousal during fear conditioning (Critchley et al., 2002). These regions are implicated in enhancement of memory of emotional material (Cahill, 2000; Cahill and McGaugh, 1998; Cahill et al., 1994).

The physiological state of our body shapes the intensity with which we experience and remember the world. This study adds to evidence for how dynamic cardiovascular signals communicate with brain to augment fear and threat responses. Stimulus processing at cardiac systole can significantly enhance fear reactivity. One implication for the understanding fear learning is that this influence of cardiac signals on the conditioned response is a potential source of variance in experimental studies of fear conditioning. Thus, the influence of cardiac timing and cardiovascular arousal should be considered in relation to task structure and stimulus presentation parameters of future fear conditioning studies. There are also implications for group comparisons, since the impact of this interoceptive channel is amplified in individuals with high trait anxiety, further influencing fear processing and subsequent fear memory. Moreover, our findings indicate that changes to the internal cardiovascular context can disrupt of block the return of fear responses, highlighting how consistency in internal bodily context contributes to preservation of such emotional memories. Together, by understanding how such embodied mechanisms give rise to persistent and recurrent fear memories, this work may help foster novel approaches targeting body-brain interactions (Watson et al., 2019) to treat fear related disorders, notably PTSD.

Context of the research

Signals from the heart were historically thought to have an inhibitory or interfering effect on stimulus processing (Lacey and Lacey, 1978). Emerging work suggests that emotional stimuli may be impervious to the inhibitory effect of the heart, and indeed that fear stimuli may even be facilitated by cardiac afferent signals (Garfinkel and Critchley, 2016). HDC has an established record of investigating mind-brain-body interactions, and SNG has additional expertise in the neural basis of persistent and recurrent fear responding in PTSD. The present

work unites these interests, to investigate how signals from the heart can augment fear learning and fear memories. This research has implications for understanding embodied mechanisms of heightened and recurrent fear in both PTSD and anxiety. Moreover, it has broader implications for psychophysiological research more generally, suggesting that variation in SCR response can be influenced by the presentation of stimuli in relation to cardiac timing. By extension, the physiological state of the individual (calm versus aroused) will affect the stability of effects across experimental sessions. To both mitigate and understand these cardiac timing effects, continuous ECG measures into future conditioning studies will allow for cardiac related SCR analyses. Understanding these variables will help to reduce noise and variance induced by fluctuations in autonomic state on SCR and fear processing.

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Author contributions

SNG, ME, TD and HDC designed the study, SLE, CDGvP, DW, ME ran experimental participants and processed data. MS processed data. SNG and ME analyzed data. All participated in the experimental write up.

Competing interests

The authors declare no competing interests.

References

Abercrombie, H.C., Speck, N.S., Monticelli, R.M., 2006. Endogenous cortisol elevations are related to memory facilitation only in individuals who are emotionally aroused. Psychoneuroendocrino 31, 187196.

Achaibou, A., 2011. Fear Memories in the Human Brain: A Functional MRI Investigation of Acquisition and Inhibition of Aversive Conditioning. Universite de Geneve.

Allen, M., Frank, D., Schwarzkopf, D.S., Fardo, F., Winston, J.S., Hauser, T.U., Rees, G., 2016. Unexpected arousal modulates the influence of sensory noise on confidence. Elife 5.

Anderson, A.K., Phelps, E.A., 2001. Lesions of the human amygdala impair enhanced perception of emotionally salient events. Nature 411, 305-309.

Azevedo, R.T., Garfinkel, S.N., Critchley, H.D., Tsakiris, M., 2017. Cardiac afferent activity modulates the expression of racial stereotypes. Nat Commun 8, 13854.

Bradley, M.M., Greenwald, M.K., Petry, M.C., Lang, P.J., 1992. Remembering Pictures - Pleasure and Arousal in Memory. J Exp Psychol Learn 18, 379-390.

Cahill, L., 2000. Neurobiological mechanisms of emotionally influenced, long-term memory. Prog Brain Res 126, 29-37.

Braithwaite, J.J., Watson, D.G., 2015. Issues surrounding the normalization and standardisation of skin conductane responses (SCRs). Technical Research Note. Selective Attention & Awareness Laboratory (SAAL), Behavioural Brain Sciences, School of Psychology, University of Birmingham.

Cahill, L., McGaugh, J.L., 1998. Mechanisms of emotional arousal and lasting declarative memory. Trends Neurosci 21, 294-299.

Cahill, L., Prins, B., Weber, M., Mcgaugh, J.L., 1994. Beta-Adrenergic Activation and Memory for Emotional Events. Nature 371, 702-704.

Critchley, H.D., Eccles, J., Garfinkel, S.N., 2013. Interaction between cognition, emotion, and the autonomic nervous system. Handb Clin Neurol 117, 59-77.

Critchley, H.D., Mathias, C.J., Dolan, R.J., 2002. Fear conditioning in humans: The influence of awareness and autonomic arousal on functional neuroanatomy. Neuron 33, 653-663.

Damasio, A.R., Tranel, D., Damasio, H.C., 1991. Somatic markers and the guidance of behavior: theory and preliminary testing, in: Levin, H.S., Eisenberg, H.M., Benton, A.L. (Eds.), Frontal lobe function and dysfunction. Oxford University Press, pp. xv,427p.

Dawson, M.E., Schell, A.M., Filion, D.L., 2017. The Electrodermal System, in: Cacioppo, J.T., Tassinary, L.G., Berntson, G.G. (Eds.), Handbook of Psychophysiology, vol. 4th Edition. Cambridge University Press, Cambridge, UK.

Do Monte, F.H., Canteras, N.S., Fernandes, D., Assreuy, J., Carobrez, A.P., 2008. New perspectives on betaadrenergic mediation of innate and learned fear responses to predator odor. J Neurosci 28, 1329613302.

Donadio, V., Kallio, M., Karlsson, T., Nordin, M., Wallin, B.G., 2002. Inhibition of human muscle sympathetic activity by sensory stimulation. J Physiol-London 544, 285-292.

Duka, T., Weissenborn, R., Dienes, Z., 2001. State-dependent effects of alcohol on recollective experience, familiarity and awareness of memories. Psychopharmacology 153, 295-306.

Dunn, B.D., Stefanovitch, I., Evans, D., Oliver, C., Hawkins, A., Dalgleish, T., 2010. Can you feel the beat? Interoceptive awareness is an interactive function of anxiety- and depression-specific symptom dimensions. Behav Res Ther 48, 1133-1138.

Dworkin, B.R., Elbert, T., Rau, H., Birbaumer, N., Pauli, P., Droste, C., Brunia, C.H.M., 1994. Central Effects of Baroreceptor Activation in Humans - Attenuation of Skeletal Reflexes and Pain Perception. P Natl Acad Sci USA 91, 6329-6333.

Edwards, L., McIntyre, D., Carroll, D., Ring, C., Martin, U., 2002. The human nociceptive flexion reflex threshold is higher during systole than diastole. Psychophysiology 39, 678-681.

Edwards, L., Ring, C., McIntyre, D., Carroll, D., Martin, U., 2007. Psychomotor speed in hypertension: Effects of reaction time components, stimulus modality, and phase of the cardiac cycle. Psychophysiology 44, 459-468.

Edwards, L., Ring, C., McIntyre, D., Winer, J.B., Martin, U., 2009. Sensory detection thresholds are modulated across the cardiac cycle: Evidence that cutaneous sensibility is greatest for systolic stimulation. Psychophysiology 46, 252-256.

Ehlers, A., Breuer, P., 1992. Increased Cardiac Awareness in Panic Disorder. J Abnorm Psychol 101, 371382.

Garfinkel, S.N., Abelson, J.L., King, A.P., Sripada, R.K., Wang, X., Gaines, L.M., Liberzon, I., 2014a. Impaired Contextual Modulation of Memories in PTSD: An fMRI and Psychophysiological Study of Extinction Retention and Fear Renewal. J Neurosci 34, 13435-13443.

Garfinkel, S.N., Barrett, A.B., Minati, L., Dolan, R.J., Seth, A.K., Critchley, H.D., 2013. What the heart forgets: Cardiac timing influences memory for words and is modulated by metacognition and interoceptive sensitivity. Psychophysiology 50, 505-512.

Garfinkel, S.N., Critchley, H.D., 2016. Threat and the Body: How the Heart Supports Fear Processing. Trends Cogn Sci 20, 34-46.

Garfinkel, S.N., Minati, L., Gray, M.A., Seth, A.K., Dolan, R.J., Critchley, H.D., 2014b. Fear from the Heart: Sensitivity to Fear Stimuli Depends on Individual Heartbeats. J Neurosci 34, 6573-6582.

Garfinkel, S.N., Seth, A.K., Barrett, A.B., Suzuki, K., Critchley, H.D., 2015. Knowing your own heart: distinguishing interoceptive accuracy from interoceptive awareness. Biol Psychol 104, 65-74.

Garfinkel, S.N., Tiley, C., O'Keeffe, S., Harrison, N.A., Seth, A.K., Critchley, H.D., 2016. Discrepancies between dimensions of interoception in autism: Implications for emotion and anxiety. Biol Psychol 114, 117-126.

Godden, D.R., Baddeley, A.D., 1975. Context-Dependent Memory in 2 Natural Environments - Land and Underwater. Brit J Psychol 66, 325-331.

Gray, M.A., Rylander, K., Harrison, N.A., Wallin, B.G., Critchley, H.D., 2009. Following One's Heart: Cardiac Rhythms Gate Central Initiation of Sympathetic Reflexes. J Neurosci 29, 1817-1825.

Hermans, D., Dirikx, T., Vansteenwegen, D., Baeyens, F., Van den Bergh, O., Eelen, P., 2005. Reinstatement of fear responses in human aversive conditioning. Behav Res Ther 43, 533-551.

Hogarth, L., Dickinson, A., Austin, A., Brown, C., Duka, T., 2008. Attention and expectation in human predictive learning: The role of uncertainty. Q J Exp Psychol 61, 1658-1668.

James, W., 1884. What is an Emotion? Mind 9.

Jeffs, S., Duka, T., 2017. Predictive but not emotional value of Pavlovian stimuli leads to pavlovian-toinstrumental transfer. Behav Brain Res 321, 214-222.

Kandasamy, N., Garfinkel, S.N., Page, L., Hardy, B., Critchley, H.D., Gurnell, M., Coates, J.M., 2016. Interoceptive Ability Predicts Survival on a London Trading Floor. Sci Rep-Uk 6.

Katkin, E.S., Reed, S.D., Deroo, C., 1983. A Methodological Analysis of 3 Techniques for the Assessment of Individual-Differences in Heartbeat Detection. Psychophysiology 20, 452-452.

Kitamura, T., Ogawa, S.K., Roy, D.S., Okuyama, T., Morrissey, M.D., Smith, L.M., Redondo, R.L., Tonegawa, S., 2017. Engrams and circuits crucial for systems consolidation of a memory. Science 356, 73-+.

Kreibig, S.D., 2010. Autonomic nervous system activity in emotion: A review. Biol Psychol 84, 394-421.

LaBar, K.S., Gatenby, J.C., Gore, J.C., LeDoux, J.E., Phelps, E.A., 1998. Human amygdala activation during conditioned fear acquisition and extinction: a mixed-trial fMRI study. Neuron 20, 937-945.

LaBar, K.S., Phelps, E.A., 2005. Reinstatement of conditioned fear in humans is context dependent and impaired in amnesia. Behav Neurosci 119, 677-686.

Lacey, B.C., Lacey, J.I., 1978. Two-way communication between the heart and the brain. Significance of time within the cardiac cycle. Am Psychol 33, 99-113.

Lewis, P.A., Critchley, H.D., 2003. Mood-dependent memory. Trends Cogn Sci 7, 431-433.

Lim, C.L., Rennie, C., Barry, R.J., Bahramali, H., Lazzaro, I., Manor, B., Gordon, E., 1997. Decomposing skin conductance into tonic and phasic components. Int J Psychophysiol 25, 97-109.

McGaugh, J.L., 2004. The amygdala modulates the consolidation of memories of emotionally arousing experiences. Annu Rev Neurosci 27, 1-28.

McIntyre, D., Edwards, L., Ring, C., Parvin, B., Carroll, D., 2006. Systolic inhibition of nociceptive responding is moderated by arousal. Psychophysiology 43, 314-319.

McIntyre, D., Kavussanu, M., Ring, C., 2008. Effects of arterial and cardiopulmonary baroreceptor activation on the upper limb nociceptive flexion reflex and electrocutaneous pain in humans. Pain 137, 550-555.

Milad, M.R., Orr, S.P., Pitman, R.K., Rauch, S.L., 2005. Context modulation of memory for fear extinction in humans. Psychophysiology 42, 456-464.

Milad, M.R., Wright, C.I., Orr, S.P., Pitman, R.K., Quirk, G.J., Rauch, S.L., 2007. Recall of fear extinction in humans activates the ventromedial prefrontal cortex and hippocampus in concert. Biol Psychiat 62, 446-454.

Naring, G.W.B., Vanderstaak, C.P.F., 1995. Perception of Heart-Rate and Blood-Pressure - the Role of Alexithymia and Anxiety. Psychother Psychosom 63, 193-200.

Norrholm, S.D., Jovanovic, T., Vervliet, B., Myers, K.M., Davis, M., Rothbaum, B.O., Duncan, E.J., 2006. Conditioned fear extinction and reinstatement in a human fear-potentiated startle paradigm. Learn Mem 13, 681-685.

O'Connell, R.G., Bellgrove, M.A., Dockree, P.M., Lau, A., Fitzgerald, M., Robertson, I.H., 2008. Self-Alert Training: Volitional modulation of autonomic arousal improves sustained attention. Neuropsychologia 46, 1379-1390.

Okuda, S., Roozendaal, B., McGaugh, J.L., 2004. Glucocorticoid effects on object recognition memory require training-associated emotional arousal. P Natl Acad Sci USA 101, 853-858.

Orr, S.P., Metzger, L.J., Lasko, N.B., Macklin, M.L., Peri, T., Pitman, R.K., 2000. De novo conditioning in trauma-exposed individuals with and without posttraumatic stress disorder. J Abnorm Psychol 109, 290-298.

Paulus, M.P., Stein, M.B., 2006. An insular view of anxiety. Biol Psychiat 60, 383-387.

Phelps, E.A., LeDoux, J.E., 2005. Contributions of the amygdala to emotion processing: From animal models to human behavior. Neuron 48, 175-187.

Pollatos, O., Traut-Mattausch, E., Schroeder, H., Schandry, R., 2007. Interoceptive awareness mediates the relationship between anxiety and the intensity of unpleasant feelings. J Anxiety Disord 21, 931-943.

Rachman, S., 1989. The Return of Fear - Review and Prospect. Clin Psychol Rev 9, 147-168.

Rashid, A.J., Yan, C., Mercaldo, V., Hsiang, H.L.L., Park, S., Cole, C.J., De Cristofaro, A., Yu, J., Ramakrishnan, C., Lee, S.Y., Deisseroth, K., Frankland, P.W., Josselyn, S.A., 2016. Competition between engrams influences fear memory formation and recall. Science 353, 383-387.

Rau, H., Elbert, T., 2001. Psychophysiology of arterial baroreceptors and the etiology of hypertension. Biol Psychol 57, 179-201.

Rescorla, R.A., Heth, C.D., 1975. Reinstatement of fear to an extinguished conditioned stimulus. J Exp Psychol Anim Behav Process 1, 88-96.

Schiller, D., Cain, C.K., Curley, N.G., Schwartz, J.S., Stern, S.A., Ledoux, J.E., Phelps, E.A., 2008. Evidence for recovery of fear following immediate extinction in rats and humans. Learn Mem 15, 394-402.

Schulz, A., Reichert, C.F., Richter, S., Lass-Hennemann, J., Blumenthal, T.D., Schachinger, H., 2009. Cardiac modulation of startle: Effects on eye blink and higher cognitive processing. Brain Cognition 71, 265271.

Spielberger, C.D., Gorsuch, R.L., Lushene, R., Vagg, P.R., Jacobs, G.A., 1983. Manual for the State-Trait Anxiety Inventory. Consulting Psychologists Press, Palo Alto, CA.

Wallin, B.G., 2007. Interindividual differences in muscle sympathetic nerve activity: a key to new insight into cardiovascular regulation? Acta Physiol 190, 265-275.

Watson, D.R., Garfinkel, S.N., van Praag, C.G., Willmott, D., Wong, K., Meeten, F., Critchley, H.D., 2019. Computerized Exposure Therapy for Spider Phobia: Effects of Cardiac Timing and Interoceptive Ability on Subjective and Behavioral Outcomes. Psychosom Med 81, 90-99.

Wiens, S., Mezzacappa, E.S., Katkin, E.S., 2000. Heartbeat detection and the experience of emotions. Cognition Emotion 14, 417-427.