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Journal Article

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Publication date:
2023-12

Permanent link:
https://doi.org/10.3929/ethz-b-000623146

Originally published in:
Psychophysiology 60(12), https://doi.org/10.1111/psyp.14386

Received: 7 February 2022 | Revised: 2 June 2023 | Accepted: 24 June 2023

DOI: 10.1111/psyp.14386

ORIGINAL ARTICLE

PSYCHOPHYSIOLOGY SPR WILEY

Cardiac interoception in infants: Behavioral and neurophysiological measures in various emotional and self-related contexts

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Funding information
Schweizerischer Nationalfonds zur Förderung der Wissenschaftlichen Forschung, Grant/Award Number: PP00P1_170511 and PP00P1_202674

Abstract

Interoception, the perception of internal bodily signals, is fundamental to our sense of self. Even though theoretical accounts suggest an important role for interoception in the development of the self, empirical investigations are limited, particularly in infancy. Previous studies used preferential-looking paradigms to assess the detection of sensorimotor and multisensory contingencies in infancy, usually related to proprioception and touch. So far, only one recent study reported that infants discriminated between audiovisual stimuli presented synchronously or asynchronously with their heartbeat. This discrimination was related to the amplitude of the infant's heartbeat evoked potentials (HEP), a neural correlate of interoception. In the current study, we measured looking preferences between synchronous and asynchronous visuocardiac (bimodal), and audiovisuocardiac (trimodal) stimuli as well as the HEP in conditions of different emotional contexts and with different degrees of self-relatedness in a mirror-like setup. While the infants preferred trimodal to bimodal stimuli, we did not observe the predicted differences between synchronous and asynchronous stimulation. Furthermore, the HEP was not modulated by emotional context or self-relatedness. These findings do not support previously published results and highlight the need for further studies on the early development of interoception in relation to the development of the self.

KEYWORDS
bodily self development, contingency, EEG, heartbeat evoked potential, self-perception

1 | INTRODUCTION

Interoception, the sense of the physiological state of the body (Craig, 2002), is both fundamental to the maintenance of homeostasis (Petzschner et al., 2021) and linked to cognition, emotion, and the sense of self (Azzalini et al., 2019). Theoretical accounts attribute an important role to interoception in the early development of self-awareness and social cognition, stating that awareness of one's own internal bodily signals might give rise to self-regulatory behaviors and social interactions necessary for the maintenance of homeostasis in early life (Ciaunica & Crucianelli, 2019; Filippetti, 2021; Fotopoulou & Tsakiris, 2017; Montirosso & McGlone, 2020; Mundy &

Jarrold, 2010). It is thought that the early development of self-awareness, which requires a distinction between the self and the other or the environment, relies on the perception and integration of contingent multisensory information (Bahrick, 2013; Jacquey et al., 2020). It has been shown that infants are sensitive to various multimodal contingencies between the environment and their own body, such as visuo-tactile, and visuo-proprioceptive synchrony from early on (Bahrick & Watson, 1985; Filippetti et al., 2013; Zmyj et al., 2011). Along these lines, it has been suggested that infants also detect contingencies between cardiac and audiovisual signals (Maister et al., 2017).

In adults, a close link between interoception and self-awareness has been proposed (Tsakiris, 2017) and empirical studies showed that focusing on oneself, for example, by looking in the mirror or at a picture of oneself, improves performance in a heartbeat tracking task (Ainley et al., 2012, 2013). Interoceptive sensitivity, the ability to accurately perceive visceral sensations, is a trait that differs between individuals and has been associated with a wide range of psychological disorders, and processes related to emotion and decision making in healthy individuals (Critchley & Harrison, 2013). Similar to the behavioral measures of interoception, the neurophysiological heartbeat evoked potential (HEP) has been found to be modulated in response to self-related (Babo-Rebelo et al., 2019; Petzschner et al., 2019), as well as emotional (Gentsch et al., 2019; Luft & Bhattacharya, 2015) stimuli. The HEP is a cortical potential that occurs 200–650 ms after the R-peak in frontocentral regions, and is thought to reflect interoceptive processing at a cortical level (Coll et al., 2021; Park & Blanke, 2019). Alterations in HEP have also been found in several clinical conditions related to both alterations of the self (e.g., Schulz et al., 2015), and of emotional processing (e.g., Flasbeck et al., 2020). Developmental investigations of the HEP are still limited, but evidence for a relation of the HEP with both a behavioral measure of interoceptive sensitivity, and emotional processing in infants has been reported (Maister et al., 2017). More specifically, the HEP amplitude was larger when infants observed negative as compared to neutral or positive emotions. Additionally, the HEP amplitude positively correlated with interoceptive sensitivity, defined as the absolute proportional difference in looking time between synchronous and asynchronous audiovisuocardiac stimuli.

In the current study, we investigated the relation between cardiac interoception, emotion, and early self-awareness. With this, we aimed to conceptually replicate and extend the previous findings by Maister et al. (2017). Similar to this previous study, we assessed interoceptive sensitivity using looking-time measures to external stimuli presented in or out of synchrony with the infant's heartbeat in the first part of the experiment. We expected previously found longer looking times for asynchronous stimulation. To further assess whether cardiac discrimination ability depends on the sensory modality of the contingency and the richness of the sensory information, we compared looking times between synchronous and asynchronous stimuli in a trimodal (audiovisuocardiac) condition, as in the original study, and added a bimodal (visuocardiac) condition, to test whether redundant sensory information is indispensable to contingency detection in a cardiac interoception task. Bimodal visuocardiac stimuli have been used previously to alter self-awareness in adults as a manipulation of contingencies between interoceptive and exteroceptive signals (Aspell et al., 2013; Heydrich et al., 2018). We thus expected bimodal visuocardiac stimulation to induce the same effects as trimodal audiovisuocardiac stimulation. In line with previous studies, we formulated the following expectations: First, we expected the infants to look longer in the trimodal condition than the bimodal condition because infants have been shown to be more interested and learn better from intersensory redundancy (Bahrick & Lickliter, 2000, 2012). Second, we expected infants to look longer toward asynchronous than synchronous stimuli (Maister et al., 2017). We had no specific expectations about a potential interaction of synchrony and modality, but such interaction would suggest that redundancy additionally affects synchrony detection.

In the second part of the experiment, which was conducted on a different day with the same participants, we concurrently measured ECG and EEG to assess the HEP in contexts varying either in emotion or self-relatedness. The aim was twofold again: First, we wanted to conceptually replicate the previously found alteration in the HEP in infants depending on the emotional context (Maister et al., 2017). Second, we aimed to test whether a modulation of the HEP could be found by alteration of self-related contexts. Emotional context has previously been shown to modulate interoceptive processes (Critchley & Garfinkel, 2017), for example through modulation of the HEP both in adults (e.g., Gentsch et al., 2019), as well as in infants (Maister et al., 2017). The latter showed that the HEP amplitude was larger when infants observed videos of faces expressing negative, as compared to positive or neutral emotions. We thus presented angry compared to happy faces and expected larger HEP amplitudes when infants looked at angry faces. In line with the previous study, we also expected a positive correlation of cardiac discrimination, quantified as the absolute proportional difference in looking time between synchronous and asynchronous stimuli, with the HEP amplitude averaged across all experimental conditions.

To extend the theoretical discussion on how interoception relates to perception of exteroceptive multisensory contingencies, we extended the EEG paradigm with a self-related context. Here, we displayed synchronous and asynchronous webcam recordings of the infant, as infants have shown to be sensitive to visuoproprioceptive contingencies from at least 4-months old (Rochat & Striano, 2002). It is thought that this awareness of multisensory contingencies is an implicit precursor to explicit mirror self-recognition between 18 and 24 months (Amsterdam, 1972; Rochat & Botto, 2021). This is further corroborated by behavioral findings showing that toddlers first pass the mirror self-recognition task exclusively when mirror displays are synchronous, and only later pass it when the display is asynchronous (Miyazaki & Hiraki, 2006). In adults, self-observation in a mirror has been shown to increase interoceptive sensitivity (Ainley et al., 2012), and the HEP was differently modulated when thinking about the self as compared to others (Babo-Rebelo et al., 2019). We thus assumed that self-observation with visuomotor synchrony, would enhance basic self-awareness and self-other distinction in infants, even before the onset of explicit mirror self-recognition. In turn, we expected this to be reflected in an increased HEP amplitude, in synchronous as compared to asynchronous videos. Finally, we assessed how interoception, as measured behaviorally and neurophysiologically, relates to infant's temperament, as measured with the IBQ-R (Putnam et al., 2014), to explore whether there is a relation between behavior and interoception already at five months of age. Furthermore, for the originally intended longitudinal design of this study, we aimed to exploratively test whether temperament predicts and is predicted by interoception throughout early development. In adults, a correlation between interoception and temperament in clinical conditions has been suggested (Lyyra & Parviainen, 2018), and infant temperament might be predictive of the development of clinical conditions, such as anxiety disorder, at a later age (Rapee et al., 2009).

2 | METHOD

2.1 | Participants

Behavioral data of 42 infants aged between 5 to 7 months were recorded, of which 31 infants (17 female, age $M = 186$ days, $SD = 15$ days) were included in the final dataset for the behavioral task. The other 11 infants were excluded because they became fussy before or during the task and did not complete enough trials (see inclusion criteria below). For the EEG session, 3 appointments had to be canceled because of a measles outbreak at the department, and 1 because of illness, meaning that 38 participants returned for the second session. Out of these, three datasets were excluded because of technical issues during the recording, 5 because of fussiness during or after application of the EEG cap, which yielded a final EEG sample of 29 infants (15 female, age $M = 191$ days, $SD = 16$ days). In total, 22 infants (11 female, age $M = 188$ days, $SD = 17$ days) provided useable data for both the behavioral and EEG session needed for the correlational analyses. Infants were recruited via a database of parents who volunteered to participate in developmental studies. All were born full term (gestational age ≥ 37 weeks) and had normal birth weight (≥2500 g). No power calculation was performed. Instead, the sample size was based on a previous study with a similar paradigm (Maister et al., 2017), while accounting for potential dropouts in a longitudinal design. Here, only a single timepoint is reported as we could not complete the longitudinal design due to the COVID-19 pandemic.

Infants received a certificate and gift worth approximately CHF 5,- as compensation for participation. Parents provided informed consent prior to participation of the study. The study was approved by the Cantonal Ethics Committee of Zurich (NR: 2018-00485) and was conducted in accordance with the ethical standards of the Declaration of Helsinki.

2.2 | Procedure

The experiment was initially planned as a longitudinal study testing children at the age of 6, 18, and 24 months. However, due to COVID-19, the study had to be interrupted and only the first timepoint was recorded. These data were collected between September 2018 and May 2019. The experiment thus consisted of two sessions, which were maximally 7 days apart. The first session consisted of the looking-time task, the second session of the EEG recording.

2.2.1 | Looking-time task

This task followed a 2×2×2 mixed design. Modality, either bimodal (visuocardiac) or trimodal (audiovisuocardiac), and Synchrony, either synchronous or asynchronous, were manipulated within participants. Finally, Speed of the asynchronous stimuli, faster (110% of the recorded heartbeat) or slower (90%) was manipulated between participants to avoid exclusively measuring a preference for speed rather than for contingency. Even though speed was counterbalanced initially, it was not balanced in the final dataset anymore due to exclusion of trials (see Table 1). Speed did not affect looking times

TABLE 1 Count of included trials for speed and modality.

	Speed		
	Slow	**Fast**	**Synchronous**
Bimodal	59	21	80
Trimodal	66	22	88

Note: In total, 336 trials were included in the looking-time analyses.

(see Supplementary Material). Modality was presented in a blocked design, with either bimodal or trimodal first in counterbalanced order. Each condition consisted of maximally six trials, where each trial was a consecutive presentation of a synchronous display and an asynchronous display (see Figure 1). The order of synchrony was counterbalanced within each modality condition, where the first set of three trials started with the synchronous display followed by the asynchronous display and the second set of three trials started with asynchronous, or vice versa. A set of twelve stimuli, each with a different color and geometric shape, was used for the task. Stimuli were grouped in sets of two, where one was assigned to synchronous displays and one to asynchronous displays throughout the task. Each stimulus appeared exclusively in either the bimodal or trimodal condition. If the full task with twelve trials was completed, each stimulus would have appeared maximally two times. Each trial was followed by the grasping preference test. The conditions were stopped early in case the infant became fussy or completely lost attention. Infant's behavior and the screen were video recorded with three cameras for later offline analyses. One camera was placed directly above the screen to record the direction of gaze, a second one filmed the infant from the top, to assess grasping responses. A third camera was directed toward the screen, to be able to synchronize the looking-time coding with the stimulus presentation.

Stimulus presentation

During the looking-time task, colored geometric shapes were presented on a computer screen placed approximately 50 cm in front of the infant, who was seated on the parent's lap. Stimulus presentation was synchronized with the heartbeat. To this end, ECG was recorded with a Biopac MP150 and ECG100C amplifier (Biopac Systems Inc, Goleta, CA, USA) from 3 Ag/AgCl electrodes (Servoprax GmBh, Wesel, Germany) placed on the infant's chest and abdomen. Data were stored using Acqknowledge software at a sampling rate of 500 Hz (Biopac Systems Inc, Goleta, CA, USA). The built in R-wave detection mode of the ECG100C amplifier was used, which detects R-peaks online and outputs smoothed R-wave peaks. This mode is more robust against movement artifacts than a complete ECG recording would be. Each time an R-wave was detected, a parallel-port trigger was sent from the Biopac to the stimulus presentation computer. This trigger was detected in MATLAB and elicited a stimulus presentation. Each trial started with an auditory attention grabber, saying "lueg emol", the Swiss German word for "look". After 1 s, the stimulus presentation started for a duration of 20 s. Pictures of the geometric shapes were presented at 5 exposure levels (−50%, −25%, 0% 25%, 50% of the original exposure of the photograph). In 100 ms, the five pictures were presented consecutively starting with the −50% to the 50%, and back to −50%, resulting in a visual blink. In the trimodal condition, the presentation of the blinks was accompanied by an acoustic sound signal, a beep with a duration of 100 ms.

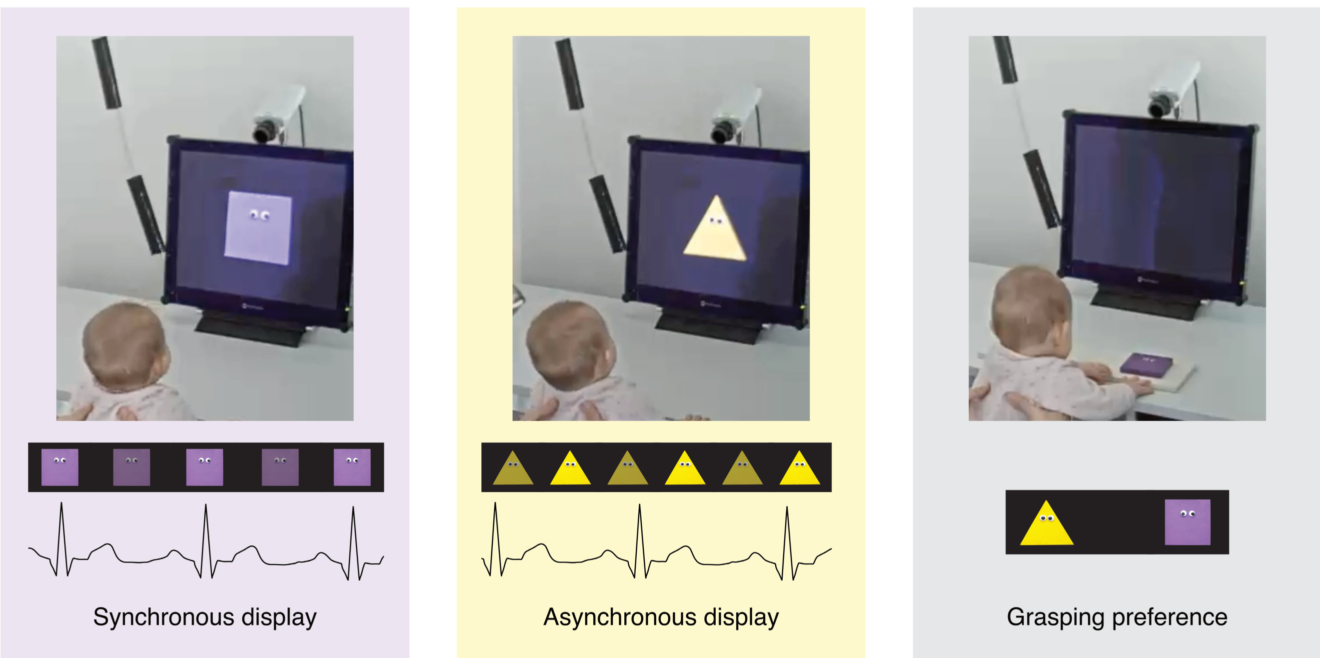

FIGURE 1 Procedure of the behavioral task. Infants were seated in front of the screen and were presented with stimuli that blinked in synchrony with the heartbeat (purple) or in asynchrony (yellow). After a synchronous and asynchronous stimulus presentation, there was a grasping trial where the two figures were presented on a wooden board in front of the infant, and the infant reached for either stimulus.

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The blinks were triggered by the ECG R-peak in the synchronous condition. In the asynchronous display, the speed of the asynchronous blinking was determined by a prerecording of three interbeat intervals, preceding the onset of each stimulus display. Then, the average of three interbeat intervals was calculated and multiplied by 0.9 (for faster) or 1.1 (for slower) to determine the speed of the blinking in the asynchronous display. R-peaks were recorded immediately before the asynchronous display to base the asynchronous presentation on the infant's heartbeat as close as possible to the onset of stimulus presentation, accounting for natural fluctuations in the heartrate. After the synchronous and asynchronous stimulus both finished, the same figures as were shown on the screen were presented in a wooden 3D model front of the infant on a wooden board to assess grasping preference. Grasping preference are not reported here due to a flaw in the setup that we only became aware of during the data analysis process. The stimuli were always presented from the same side, biasing grasps strongly toward the first stimulus that came into the infant's field of view.

Measures

In the looking-time task, the preference for synchronous or asynchronous stimuli was assessed by the infants' looking preference. Looking time was coded as the time that the infant's gaze was directed at the screen while the stimuli were presented on screen.

2.2.2 | EEG task

EEG recordings were performed in a dimly lit, sound attenuated, and electrically shielded room. The infants were seated on the lap of the caregiver at approximately 60 cm from a 17-inch monitor. A black cloth covered the caregivers' body, as to mask out any movements from the caregiver. The infant was filmed with a webcam, which was positioned so that the infants full face and body were in view, but the caregiver's face was out of view. In counterbalanced order, three blocks of each emotional condition (happy and angry), and three blocks of each self-related condition (self-synchronous and self-asynchronous) were presented (Figure 2). Emotional faces were taken from the Montréal Pain and Affective Face Clips (Simon et al., 2008). Clips from four female and four male actors were selected. Each face appeared on the screen for 2 s, where the emotion was displayed at 50% intensity, then gradually changed to 100%, and back to 50%. Videos for the happy and angry condition were edited to a length of 20 s. For the self-synchronous condition, the recording from the webcam was displayed on the screen in real time for 20 s. For the self-asynchronous condition, the recording was delayed with 3 s and then played back for 20 s.

Measures

The EEG was recorded from a 128-channel Geodesic Sensor Net with a NetAmps 300 amplifier (Electrical Geodesics Inc., Eugene, OR, USA, Figure 3) at a sampling rate of 500 Hz. Data were recorded with reference to the vertex and online bandpass filtered between 0.01 and 100 Hz. The impedance of all electrodes was kept below 50 kΩ. Concurrent ECG was recorded with a 2-electrode setup, one on the lowest left rib, one on the right clavicle, with a Polygraph Input Box (Electrical Geodesics Inc., Eugene, OR, USA) connected to the same NetAmps 300 amplifier.

Looking time during stimulus presentation was assessed with two cameras, one filming the infant's face, and another one filming the stimuli on the screen. Looking duration was coded as the time that the infant's gaze was directed to the screen.

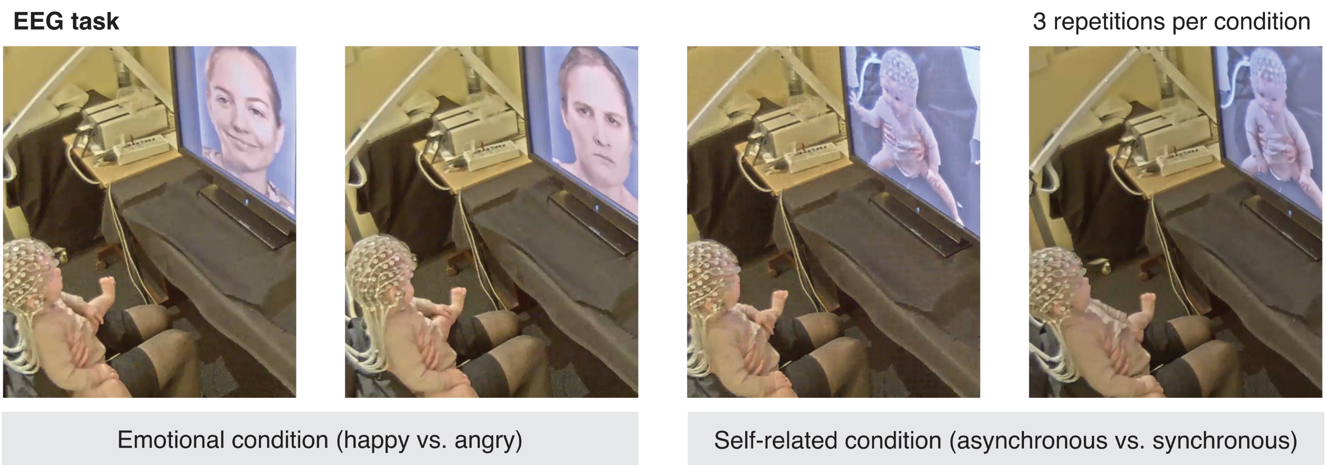

FIGURE 2 Procedure and stimuli of the EEG task. Infants were seated on the parent's lap in front of a display. They saw moving happy and angry faces in the emotional conditions, and real-time or delayed videos of themselves in the self-related condition. Each stimulus lasted for 20 s and was repeated maximally three times.

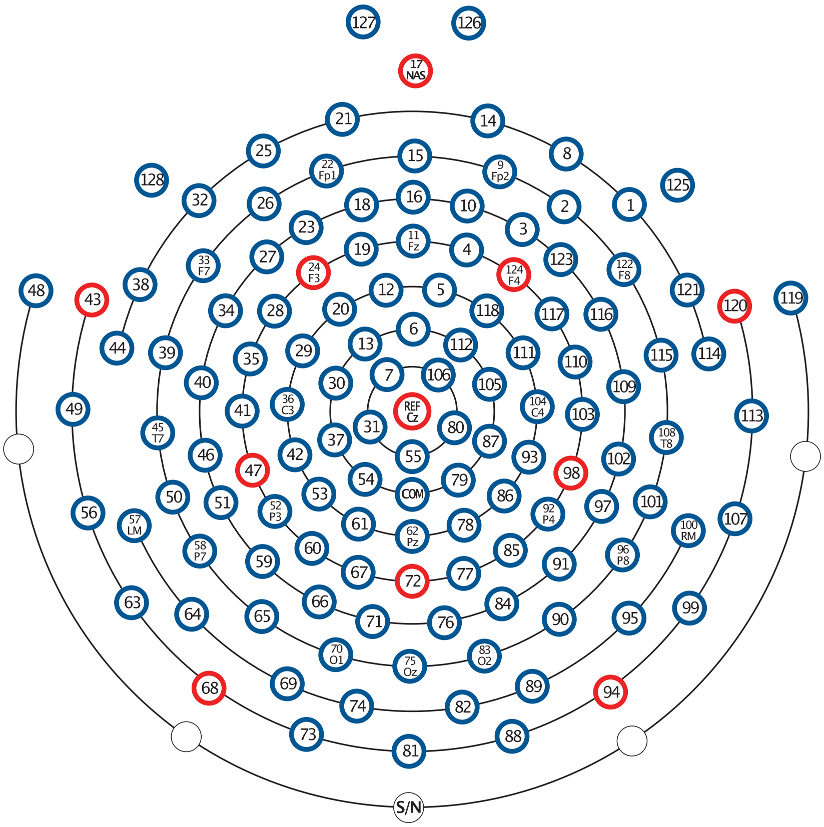

FIGURE 3 Channel layout of the 128-channel Geodesic Sensor Net. The outer electrodes (E43, E48, E49, E56, E63, E68, E73, E81, E88, E94, E99, E107, E113, E119, E120, E125, E126, E127, E128) were removed from the analyses.

2.2.3 | Infant behavior questionnaire

At the end of the first session, caregivers completed the Infant Behavior Questionnaire—Revised—Very Short Form (IBQ-R), a 37-item questionnaire that assessed three broad subscales of infant temperament (Putnam et al., 2014). Responses were given on a 7-point Likert scale, and subscale scores for Surgency, Negative Affect, and Effortful Control were calculated as the mean over the items belonging to each subscale.

2.3 | Data processing

2.3.1 | Looking-time preprocessing

As a first step, the ECG and stimulus presentation during the looking-time task were assessed to include trials. For synchronous displays, the ECG had to match with 85% of the recorded R-peaks. In synchronous displays, this meant that sometimes a beat was skipped, because no R-peak was detected, and thus, the stimulus did not light up. In asynchronous displays, if the speed was set to 110%, the displays had to be between 85% and 130% of the actual heartrate, and if the speed was set to 90%, the displays had to be between 70% and 115% of the actual heartrate. These percentages were chosen based on the errors that could occur in the recording of three interbeat intervals at the beginning of the asynchronous display. Important to note is that a speed of 100% in the asynchronous display meant that just the speed was similar as the recorded heartrate, but the presented beats still did not coincide with the recorded R-peaks, unlike in the synchronous displays.

In the second step, valid trials were selected based on the monitoring videos. Looking time and other behavior during stimulus presentation was manually coded by the first author, who was not blind to the conditions. A second observer, who was not aware of the hypotheses of this study, independently coded the videos of 25% of the participants. The intraclass correlation coefficient based on a single-rating, 2-way mixed-effects model indicated excellent reliability between the two raters (ICC = 0.96, 95% CI = [0.95, 0.97]). Trials were excluded if there were distractions from the parent, experimenter, or other reasons in the room or if the infant cried for more than 5 s. A trial was included if both the synchronous and asynchronous stimulus presentation met all the criteria above. 148 single stimulus presentations had to be excluded because there was no corresponding synchronous, or asynchronous stimulus in the same trial. A modality condition was included when at least one full trial was completed. This resulted in an average inclusion of 3.0 ± 2.1 trials in the bimodal condition, and 3.3 ± 2.1 trials in the trimodal condition per participant (see Table 1 for the count of trials in each condition).

2.3.2 | Heartbeat evoked potentials preprocessing

EEG and ECG recordings were preprocessed using Matlab 2017b and EEGLAB (version 14.1.1b; Delorme & Makeig, 2004). The outer electrodes (E43, E48, E49, E56, E63, E68, E73, E81, E88, E94, E99, E107, E113, E119, E120, E125, E126, E127, E128, Figure 3) were removed from the data due to bad contact with the scalp. R-peaks in the ECG were detected with the EEGLAB extension HEPLAB (Perakakis, 2019) using the ECGLAB slow algorithm (de Carvalho et al., 2002). Automatic R-peak detection was followed by a visual inspection and manual correction to ensure appropriate R-peak detection. EEG data were filtered with a 0.3–30 Hz bandpass filter. Bad channels were detected using visual inspection and replaced using spherical interpolation. The data were re-referenced to the average reference. Afterwards, data were segmented in 20 s long segments, that corresponded to the four conditions. Epochs from −100 to 350 ms relative to the R-peak were extracted for each condition. No baseline correction was performed, to avoid both artifacts from preceding heartbeats, and contaminations from potential late components of the preceding HEP (Petzschner et al., 2019). Epochs were rejected using a semiautomatic approach. First, all epochs containing multiple R-peaks (RR interval < 350 ms) were rejected, as to avoid contamination with the cardiac field artifact of the subsequent heartbeat. Then, epochs where one or more electrodes exceeded an amplitude of ±250 μV were rejected. Finally, the datasets were visually inspected for further artifacts. For statistical analyses, a grand average over all epochs within each condition was calculated (see Table 2 for the number of epochs included in each condition).

3 | RESULTS

3.1 | Behavioral task

A Bayesian multilevel model was used to analyze the looking-time data. The model was implemented in Stan (Carpenter et al., 2017) using the R-package brms (Bürkner, 2017). Samples of posterior probability distributions for all estimated parameters were drawn with a Hamiltonian Monte Carlo sampling algorithm. Four independent Markov chains, each with 1000 warm-up samples, followed by 3000 samples from the posterior distribution were used. The final 3000 samples of each chain were used for statistical inference. Minimally informative priors were used for all parameters of the model (normal distribution with $M = 0$, and $SD = 10$). Convergence was confirmed with R hat statistics that were < 1.1 for all estimates. For the Bayesian multilevel model, a random intercept was modeled for each participant. Additionally, main effects synchrony (synchronous vs. asynchronous) and modality (bimodal vs. trimodal) were modeled. The model was specified as following: (lookingtime ~ synchrony + modality + synchrony × modality + (1|participant.ID)). We used a region of practical equivalence (ROPE) procedure for hypothesis testing (Harms & Lakens, 2018). The ROPE is defined as [−0.1 × SDy, 0.1 × SDy], where y is the parameter of interest (Kruschke, 2018). The alternative hypothesis is accepted if the 95% credible interval (CI) of the posterior distribution of the model parameter falls completely outside the ROPE. If the 95% CI falls completely inside the ROPE, the null hypothesis is accepted. A partial overlap of the 95% CI and ROPE is interpreted as inconclusive evidence.

The Bayesian multilevel model showed no conclusive effect of synchrony on looking time ($b = -0.47$, 95% CI = [−1.71, 0.79], 45.8% in ROPE). There was an effect of modality ($b = -2.40$, 95% CI = [−3.73, −1.12], 0% in ROPE), showing that infants looked longer at trimodal ($MPE = 10.87$, 95% CI = [9.96, 11.80]) than bimodal stimuli ($MPE = 9.06$, 95% CI = [8.15, 10.10], Figure 4). Evidence for the interaction of modality and synchrony was inconclusive ($b = 1.20$, 95% CI = [−0.64, 2.99], 18.9% in ROPE).

In the bimodal condition, 16 infants looked longer at synchronous, and 11 infants longer at asynchronous stimuli. Analogous with the looking time analyses above, we did not find conclusive evidence for a higher frequency of longer looking times at synchronous stimuli (0.59) being different from chance with a Bayesian binomial test (95% CI = [0.41, 0.76], 7.2% in ROPE). In the trimodal condition, 13 infants looked longer at synchronous, and 14 infants longer at synchronous stimuli, the binomial test again showed no conclusive evidence that this was different from chance (estimated frequency of longer looking times at synchronous stimuli: 0.48; 95% CI = [0.30, 0.66], 11.1% in ROPE). The ROPE for binomial tests was defined as [0.4875, 0.5125], which is half of an effect with a small effect size.

Twenty-three out of 31 infants provided useable data for both conditions. From these, 12 infants demonstrated the same synchrony preference for the bimodal and trimodal condition (5 preferred asynchronous and 7 synchronous stimuli). Out of the 11 infants who showed different

TABLE 2 Epoch count per condition.

Condition	Mean number of epochs (*SD*)
Angry	70.3 (22.2)
Happy	63.0 (28.3)
Self-synchronous	55.4 (25.6)
Self-asynchronous	69.2 (32.9)

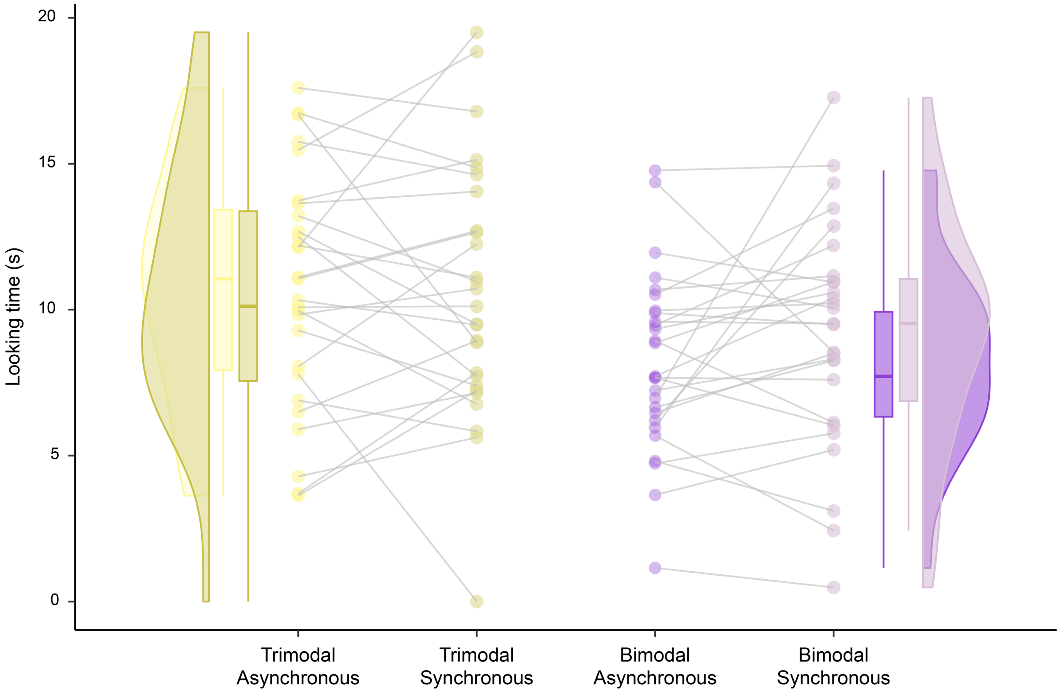

FIGURE 4 Raincloud plots of looking time in each condition. There was a significantly longer looking time in the trimodal than the bimodal conditions. Colors indicate the different conditions and dots the individual observations. Boxes indicate the interquartile ranges, horizontal lines mark the medians, and whiskers indicate the lower and upper extremes.

TABLE 3 Responses to the IBQ-R.

IBQ-R subscale	Mean (*SD*)
Surgency	4.91 (0.68)
Negative affect	3.60 (0.96)
Effortful control	4.82 (0.59)

TABLE 4 Results of the Bayesian correlation analyses of interoceptive sensitivity with the IBQ-R.

IBQ-R subscale	Rho	95% CI	BF_{10}	% in ROPE
Surgency	0.14	−0.20, 0.45	0.56	17.1
Negative affect	−0.15	−0.46, 0.19	0.59	16.4
Effortful control	0.10	−0.24, 0.42	0.47	20.4

synchrony preferences between modalities, 8 preferred asynchronous stimuli in the trimodal condition, and synchronous stimuli in the bimodal condition, and 3 infants preferred asynchronous stimuli in the bimodal condition, and synchronous stimuli in the trimodal condition.

We furthermore assessed whether interoceptive sensitivity, defined as the absolute proportional difference in looking time between synchronous and asynchronous stimuli ($M=0.110$, $SD=0.105$) was correlated with the subscales of the IBQ-R (responses to each of the IBQ-R subscales are summarized in Table 3). To this end, we performed Bayesian correlation analyses using the BayesFactor package in R. All of the three subscales of the IBQ-R showed inconclusive evidence for a correlation with interoceptive sensitivity (Table 4; correlations are plotted in Figure S1 of the Supplementary Material).

3.2 | HEP

As the HEP is a cortical potential that has been reported to be highly heterogeneous in terms of topography and latency (Coll et al., 2021; Park & Blanke, 2019), we performed cluster-based permutation *t*-tests implemented in FieldTrip (Maris & Oostenveld, 2007; Oostenveld et al., 2011) to assess for any differences between experimental conditions, while controlling for multiple comparisons across space and time. This procedure clusters adjacent spatiotemporal data points for which *t*-values exceeded a cluster threshold *p*-value of .05 (two-sided). Cluster statistics are calculated as the sum of the *t*-values of all points within a spatiotemporal cluster. This cluster statistic was then evaluated under the cluster-distribution under the null hypothesis. Condition labels were randomly shuffled 1000 times, and maximum cluster-level statistics were retained for each permutation. The two-tailed Monte Carlo *p*-value reflects the proportion of elements in the distribution that exceeded the observed maximum cluster-level statistic and was considered significant <.05. Neighbors distance of 4 cm, on average 6.3 neighbors per channel. Cluster based permutation *t*-tests did not show any significant differences in the HEP between conditions for both the emotional context and the self-related context.

Additional control analyses, to assess whether looking duration differed between the conditions in the EEG task, using Bayesian multilevel models with the same parameters as described above, did not show conclusive evidence for a difference in looking time between angry and happy faces ($b = -0.41$, 95% CI = [−1.77, 0.96], 51.3% in ROPE). Neither was there a conclusive evidence for a difference in looking time between the self-synchronous, and self-asynchronous conditions ($b = -0.12$, 95% CI = [−1.26, 1.02], 61.8% in ROPE; looking durations are reported in Table 5).

Finally, to assess whether HEP amplitude, independent of experimental condition correlated with any of the subscales of the IBQ-R, or interoceptive sensitivity defined as the absolute proportional looking-time difference between synchronous and asynchronous stimuli in the looking-time task, cluster-based permutation correlation analyses were performed on the HEP averaged per participant across all conditions with each of the subscales of the IBQ-R. The HEP across all conditions did not correlate with any of the subscales of the IBQ-R. Also, no significant clusters for a correlation of the HEP and interoceptive sensitivity were found.

4 | DISCUSSION

In this study, we investigated interoception and its link to emotion and self-awareness in infants aged 5–7 months. Contrary to our prediction, we did not find conclusive evidence for a systematic looking preference toward either asynchronous or synchronous stimuli, while we confirmed a clear preference for trimodal as compared to bimodal stimuli. Additionally, and again unlike predicted, we did not observe any differences in the HEP across either varying emotional or self-related contexts, nor did the HEP correlate with parents' reports of infant temperament or the individual differences in looking time. Taken together, these results do neither support our hypotheses, nor are they in line with previous evidence of interoceptive sensitivity in infants recorded in a similar experimental setup (Maister et al., 2017). We can currently only speculate about the reason for these inconclusive findings.

TABLE 5 Looking duration in each of the EEG conditions.

Condition	Mean (*SD*) in s
Angry	12.0 (5.4)
Happy	11.6 (5.3)
Self-synchronous	12.2 (5.0)
Self-asynchronous	12.3 (4.5)

In the looking-preference task, we found that infants looked longer at trimodal (audiovisuocardiac) than bimodal (visuocardiac) stimuli. This preference was not modulated by synchrony and confirms the validity of our looking-time preference measures. The availability of more sensory information led to an increase in attentional resources dedicated to these stimuli (Bahrick & Lickliter, 2000). We did however not replicate the finding that infants distinguish in looking time between stimuli that were either in- or out of synchrony with their own heartbeat (Maister et al., 2017). This lack of conclusive evidence could speculatively be linked both to subtle differences in the experimental paradigm, and to age differences.

In our study, we manually coded looking time, whereas Maister et al. (2017) used eye tracking and gaze-controlled stimulus presentation, which may have increased sensitivity to detect the small to medium effect size reported by Maister et al. (2017). We also used blinking objects, that were accompanied by a beep in the trimodal condition, contrastingly, Maister et al. (2017) used stimuli that moved up and down and were accompanied by an acoustic signal. It may have been the case that the difference between synchronous and asynchronous blinking stimuli was not salient enough to observe a looking-time difference, which however questions the generalizability of previous data. Alternatively, the use of both a bimodal and trimodal condition may have interfered with synchrony detection, even though the presentation was counterbalanced, thus masking a potential effect due to our experimental design. Furthermore, the addition of these conditions could have decreased statistical power relative to the previous study with a less complex design (Maister et al., 2017), which might be especially relevant as no formal power calculation was performed. Furthermore, the way asynchrony was determined could have led to different results. While we based asynchrony on the infant's heartrate immediately preceding the trial, Maister et al. (2017) used the heartrate of the previous trial. The immediacy of the asynchrony might have resulted in either more or less asynchrony with the infant's heartbeat during the trial, which in turn could have affected looking preference. Future studies should carefully consider the way asynchrony is determined and presented.

Another explanation could be found in the age difference between the two studies. Our sample was slightly older, which may have affected contingency detection. In paradigms using other sensory modalities discrimination ability emerges at different ages, and might even switch between a preference for contingent stimuli first, followed by a preference for non-contingent stimuli (Jacquey et al., 2020). Recent theories of interoceptive development state its importance for effective infant-caregiver interactions to maintain homeostasis (Ciaunica & Crucianelli, 2019; Filippetti, 2021; Fotopoulou & Tsakiris, 2017). This could link to the idea that while contingency detection of movement-related senses or of external stimuli (i.e., proprioception or touch) increases with increasing age (Rochat, 2003), the link between external and interoceptive signals might decrease, which could be demonstrated by decreasing attention to bodily signals. This hypothesis would be in line with the idea that when the infant grows older and motor skills develop, feeding interactions become more efficient (Filippetti, 2021), and instead attention is directed to the exploration of the world (Adolph & Hoch, 2019).

Additionally, we did not find neurophysiological evidence for a modulation of interoceptive processing in infants across emotional or self-related contexts. A potential reason for that could be that we tested for differences in HEP amplitude while altering the emotional or self-related context by visual means only, it could be the case that the stimuli used were not salient enough to observe changes in the HEP. Emotion perception appears to be driven by perception of voice rather than facial expression in early infancy (Caron et al., 1988; Flom & Bahrick, 2007). It has been demonstrated that 4-month-old infants only discriminated affective facial expression when voice and face were presented in synchrony, but not when multimodal stimuli were presented asynchronously, or unimodal auditory or visual stimuli were presented (Flom & Bahrick, 2007). Sensitivity to visual affective stimuli only emerged at 7-months. The infants tested in the current study were between 5 and 7 months old, and thus, the ability to discriminate between different emotional facial expressions from unimodal visual stimuli might not be fully developed, which could explain why we did not observe a difference in HEP. Still, this would not explain the difference to previous findings in a very similar setup using visual stimuli only (Maister et al., 2017). Nevertheless it would be interesting, to use auditory, or audiovisual emotional stimuli (Caron et al., 1988; Flom & Bahrick, 2007).

Such a similar reasoning might be the case of the self-related stimuli. Even though, 5-month-old infants have been reported to distinguish between synchronous and asynchronous displays of their leg movements (Bahrick & Watson, 1985), these results are not consistent and rapidly developing (Zmyj et al., 2009). Furthermore, while contingency perception has been discussed as an important precursor for self-recognition at a later age, these two concepts do not directly correlate (Klein-Radukic & Zmyj, 2020). Furthermore, for explicit self-recognition, which has been found to modulate interoception in adults (Ainley et al., 2012, 2013), recognition of one's own facial features seems an additional driving force in addition to multisensory contingency. Facial self-recognition only emerges later in infancy (Filippetti & Tsakiris, 2018), and therefore contingency detection alone might not directly modulate the HEP. Longitudinal studies, relating cardiac interoception in infants to development of affect and self-awareness, would be required to experimentally address whether and how these concepts relate and potentially predict behavioral outcomes. To further study how interoception relates to behavioral outcomes, future studies should address the long-term stability and reliability of interoceptive measures. In adults, poor reliability of the HEP across experimental sessions has been reported (Verdonk et al., 2021), and behavioral measures show similar variability across timepoints in children (Ferentzi et al., 2018). While such variability could partially be explained by developmental changes across childhood (Ferentzi et al., 2018), the long-term stability and reliability of these measures requires urgent investigation to better understand the developmental trajectory of interoception.

Another potential limitation might be the case that our experimental design did not provide sufficient power and a high enough signal to noise ratio to detect the effects of interest in the HEP, despite at least as many repetitions as in previous studies (Maister et al., 2017). The HEP is a highly variable neural potential, that has been found across different latencies and topographies in adults (Coll et al., 2021). Furthermore, large amount of trials, or R-peaks, up to ten times higher as in the current experiment, are recommended to obtain reliable results in adult studies (Park & Blanke, 2019). To increase the number of trials as much as possible, we opted for presenting the stimulus on screen for 20 s, without having a gaze-controlled stimulus presentation. While looking time was comparable between all conditions, we cannot ensure that the infant looked at the screen for the full 20 s period, and thus, there may have been periods in which no awareness of the self-related or emotional stimuli was present. Together with the short duration of the EEG recording, due to the limitations of experiments with infants, this might not have provided enough trials to find a reliable HEP.

4.1 | Outlook

The growing literature on interoception development emphasizes its importance in relation to development of psychopathology (Murphy et al., 2017), and highlights its role in development of self-awareness and social cognition (Ciaunica & Crucianelli, 2019; Filippetti, 2021; Fotopoulou & Tsakiris, 2017). There is however a gap between theoretical discussions of interoceptive development and empirical evidence. More studies, using larger samples would be required to assess generalizability of the inconclusive evidence reported in our study, and positive findings reported earlier (Maister et al., 2017). While assessments of interoceptive awareness at a single timepoint are a start to better understand how interoception develops, longitudinal studies and investigations addressing the reliability and long-term stability of the measures used would be required to obtain a broader understanding of the implications of interoception in early life. Aligning theory and empirical data, by investigating how interoception relates to the development of emotion regulation and self-awareness across infancy and childhood longitudinally, might provide a more complete understanding of the implications interoception in psychopathology from a developmental perspective.

AUTHOR CONTRIBUTIONS

Marieke L. Weijs: Conceptualization; data curation; formal analysis; investigation; methodology; project administration; software; visualization; writing – original draft; writing – review and editing. **Moritz M. Daum:** Conceptualization; resources; writing – review and editing. **Bigna Lenggenhager:** Conceptualization; funding acquisition; methodology; project administration; resources; supervision; writing – review and editing.

ACKNOWLEDGMENTS

We thank Marco Bleiker for his help in various study related tasks, Fiona Pugin and Peyman Khodadust for assistance with the experimental setup, Leonie Hartmann and Raphaela Schnider for help with recruitment, and Regina Anton for assistance with coding the videos. Thanks to the participants and parents for taking part in this study. Open access funding provided by Universitat Zurich.

FUNDING INFORMATION

This research was supported by the Swiss National Science Foundation (grant number: PP00P1_170511 and PP00P1_202674).

DATA AVAILABILITY STATEMENT

All stimulus materials, analysis scripts, and the preprocessed data have been made available on the Open Science Framework: https://osf.io/qgdw6/, DOI: 10.17605/OSF.IO/QGDW6.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Table S1: Summary of the Bayesian multilevel model predicting looking time by speed.
Figure S1: Correlations between looking time and IBQ subscales. There were no significant correlations between the absolute proportional difference in looking time between synchronous and asynchronous stimulation and the IBQ subscales of (a) surgency, (b) negative affect, and (c) effortful control.

How to cite this article: Weijs, M. L., Daum, M. M., & Lenggenhager, B. (2023). Cardiac interoception in infants: Behavioral and neurophysiological measures in various emotional and self-related contexts. *Psychophysiology*, *60*, e14386. https://doi.org/10.1111/psyp.14386