



Universidade do Minho
Escola de Psicologia

Sandra Cristina Carvalho Queirós

**Activation in the Right Posterior
Temporal Region of 7-Month-Olds to
Upright Point-Light Walkers: A fNIRS
Study**



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Mestrado Integrado em Psicologia

Trabalho efetuado sob a orientação do

Doutor Alfredo Pereira

Declaração

Nome: Sandra Cristina Carvalho Queirós

Endereço Eletrónico: a68783@alunos.uminho.pt

Número do Cartão de Cidadão: 14623719

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Assinatura: Sandra Cristina Carvalho Queirós

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“Science is a beautiful gift to humanity; we should not distort it.”

A.P.J.Abdul Kalam

Ativação na Região Posterior Temporal Direita, aos 7 Meses de Idade, para *Point-Light Walkers* Canônicos: Um Estudo fNIRS

Resumo

Os seres humanos são especialmente hábeis a distinguir o movimento de seres vivos dos outros tipos de movimento. Estudos desenvolvimentais indicam que a preferência pelo movimento biológico (MB) é observável desde cedo na infância. Recentemente, tem-se procurado compreender as bases neuronais associadas a este processo. Uma conclusão consistente nos estudos conduzidos com técnicas de neuroimagem e eletrofisiologia é que o Sulco Temporal Superior (STS) direito parece estar associado ao processamento de MB assim como à cognição social. No entanto, a via neuro-desenvolvimental associada a este processo não é clara. O presente estudo, conduzido com fNIRS, mediu a resposta hemodinâmica na Região Posterior Temporal direita –que inclui a STS - a um estímulo padrão em estudo do MB, *Point-Light Walkers* (PLW). Testaram-se sujeitos com sete meses de idade ($n = 11$), num design experimental intra-sujeito, com duas condições experimentais: um *PLW* canônico e (2) um *Spatially Scrambled PLW*, ambos em aproximação. Os resultados demonstraram resposta hemodinâmica ao *PLW* canônico, comparativamente ao *Spatially Scrambled PLW* na região temporal posterior direita. Estes resultados parecem confirmar os resultados anteriores e, que nesta idade, o MB é processado de uma forma diferente de outro tipo de movimento nesta região.

Palavras-Chave: Movimento Biológico (MB); Região Temporal Posterior; *functional near-infrared spectroscopy* (fNIRS); Desenvolvimento Infantil; *Point-Light Walker* (PLW)

Activation in the Right Posterior Temporal Region of 7-Months-Olds to Upright Point-Light Walkers: A fNIRS Study

Abstract

Humans are particularly good in distinguishing the movements of living beings from another type of motions. Studies conducted with children showed that the preference for biological motion (BM) can be observed early in infancy. Recently, researchers are focusing on understanding the neurological basis associated to the processing of BM. One conclusion that has been consistent across the studies conducted with neuroimage and electrophysiological techniques is that the right Superior Temporal Sulcus (STS) seems to be associated to BM's processing and with social cognition. However, the developmental path associated with this process is unclear. The present study, conducted with fNIRS, measured the haemodynamic response in right posterior temporal region – an area that includes the right posterior STS- to a standard stimuli of BM study, Point-light walkers (PLW). Seven-months-olds infants (n = 11) were tested in a within-subjects blocked design with two experimental conditions: (1) an Upright PLW and (2) a Spatially Scrambled PLW, both approaching. The results demonstrated haemodynamic response for the Upright PLW, compared to the Spatially Scrambled PLW, in the right posterior temporal region. These results are in accordance with previous findings and that, at this age, BM is processed differently from other types of motion in this region.

Key-Words: Biological Motion (BM); Right Temporal Posterior Region; functional near-infrared spectroscopy (fNIRS); Infant Development, Point-Light Walker (PLW).

Introduction

We live in a dynamic moving world and from all different kinds of motion, we seem to be particularly tuned to Biological Motion. The term Biological Motion (BM) has been coined by Gunnar Johansson to refer to the ambulatory patterns of terrestrial bipeds and quadrupeds (Hoffman & Flinchbaugh, 1982). This type of motion, is typically a semi-rigid motion: when a vertebrate moves, its limbs and torso undergo a specific pattern of non-linear motion constrained by the rigid skeletal structure of its supporting body. Spatial relationships among certain body parts are continuously changing (e.g. the motion of the arm and the motion of the hip), whereas spatial relations between other parts remain invariant, i.e: the distance between their connected joints is rigid and does not change, for example the distance between the elbow and shoulder (Bardi, Regolin, & Simion, 2011).

The seminal work of Johansson (1973) introduced a technique for studying biological motion using only minimal information: he attached small illuminated dots to the major joints of a human participant and created a Point-Light Walker (PLW); from these, the motion of a person walking could readily be identified. Johansson showed that the human visual system only needs five to ten dots in adequate combinations of proximal motion to give the vivid impression of a person walking and that we need no more than 200ms of stimuli presentation to identify a PLW (Johansson, 1973). This technique is one of the most robust and replicable methodologies used in the study of the processing of BM and has the advantage of allowing the separation of information revealed by motion from other sources of information (e.g.: form, texture, color, etc.). The Point Light Displays (PLD) technique has been largely used in studies with adults but also with infants. Canonical PLW is the most common stimulus used in this research field and consists of a PLW in an upright orientation. Most of the studies contrasted performance between canonical PLW's with manipulations, namely inversion, masking and other types of motion (random or not), using tasks of discrimination, preferential looking and force-choice paradigms. The use of PLD is important in the extent that it allows us to study the impact of specific motion properties in BM's perception: a key question in this research field is the contribution of the global motion structure, the local relation between the dots, and each individual dot's motion to BM perception (Bertenthal & Pinto, 1994).

Developmental studies, using behavioral tasks, show that visual sensitivity to BM emerges early on: by four-six months of age, infants exhibit a preference for a canonical point-light human walker over an inverted walker or a random motion (Fox & McDaniel, 1982) and by five months of age infants are sensitive to local rigidity on an upright PLW but not in inverted

PLW's (Bertenthal, Proffitt, & Kramer, 1987). In fact, more recent studies conducted with newborns found that a preference for biological motion is present at birth: one study conducted with two-day-old newborns that used a point-light hen as stimulus found a visual preference for an upright hen over a random motion or an upside-down display (Simion, Regolin, & Bulf, 2008). By nine months of age, infants discriminate a human point-light walker from similar displays with scrambled spatial relationship (Bertenthal, Proffitt, Spetner, & Thomas, 1985) which suggests that at least by that age infants detect a structure consistent with a human form in PLDs. Another experiment conducted with two-day-old infants demonstrated that newborns discriminate between a walking and a scrambled hen, however the subjects did not show any preference for the walking hen compared to the scrambled hen (Bardi et al., 2011). Additionally, another study conducted with three day-old infants demonstrated that newborns do not exhibit any preference for a PLW over random motion when both stimuli had a translational component (Bidet-Ildei, Kitromilides, Orliaguet, Pavlova, & Gentaz, 2014). The outcome of these studies suggests that since early infancy, humans are sensitive to some spatial relationships between the elements that compose biological motion although this topic is not completely understood.

In neuroimaging studies one brain area has been consistently associated with BM processing: the Superior Temporal Sulcus (STS), an area linked to social cognition (Pavlova, 2011; Troje, 2013). The study of this connection, has received important contributions from neuroimaging and electrophysiological techniques. These techniques also revealed several brain areas involved in BM processing. Grossman et al. (2006) conducted a study with functional magnetic resonance imaging (fMRI) in adults and compared neural activation elicited by BM vs Scrambled Motion, using the PLD technique. Their main finding was a single locus of activation for biological motion was found in the posterior STS (Grossman et al., 2000). Their results were corroborated by other studies, such as Vaina, Solomon, Chowdhury, Sinha, and Belliveau (2001) and Beauchamp, Lee, Haxby, and Martin (2003).

Recently, several neurodevelopmental studies have started mapping BM processing in infancy, in particular electroencephalography (EEG) studies. Reid, Hoehl, and Striano (2006) conducted one study with infants and EEG on BM's processing. Their results indicate that, by eight months of age, right parietal regions are involved in the detection and assessment of biological motion and that brain activity has a larger amplitude for a PLW with upright orientation in the right hemisphere when compared with an inverted PLW. Hirai and Hiraki (2005) tested eight-month-olds infants, using EEG, to study the neural correlates to the perception of BM when presented with upright PLW and scrambled Motion. The main from this study were: (1) during perception of BM, the average amplitude of response in the right

hemisphere was greater than in the left hemisphere; and (2) response to BM was significantly greater than to scrambled motion. The main activation observed corresponded to the T5/T6 areas (using the 10-20 system), which are in the occipital-temporal region. The results of this study appear to coincide with prior work on biological motion processing in adults, suggesting that right occipital-temporal region, including the STS, plays an important role in BM's perception. Finally, Marshall and Shipley (2009) collected event-related potential (ERP) data from five-months-old infants using a set of PLDs performing different actions. Infants were presented with two conditions of PLDs, (1) upright canonical sequences (running, walking, throwing a ball and kicking a ball) and (2) position-scrambled versions of the same sequences. They hypothesized that infants would be sensitive to the contrast between scrambled and canonical displays. Their results revealed that, at mid-parietal and occipital sites, the canonical stimuli produced a more positive-going wave-form to the scrambled stimuli. At the temporal and parietal sites, the wave-form elicited for the scrambled PLD's was more positive than the waveform to the canonical displays. In summary, this study provided converging evidence that by five months, the processing of the upright human motion is sensitive to disruptions in global form.

Infants reveal differences in the processing of upright PLW and scrambled PLW's. Behavioural studies indicate a discrimination since very early between BM, scrambled and random motion (Bertenthal, Proffitt, & Cutting, 1984; Bertenthal, Proffitt, Kramer, & Spetner, 1987; Fox & McDaniel, 1982). Neurophysiological studies also indicate different neural responses for an upright PLW vs scrambled motion in occipito-temporal region, specifically in the right hemisphere (Hirai & Hiraki, 2005; Marshall & Shipley, 2009). These observations suggest that global motion structure is important for BM processing. The STS, localized in the temporal area, seems to play an important role for the BM processing to. However, EEG studies have a poor spatial resolution making localization and in particular determining if STS is involved in BM processing difficult.

Another (relatively novel) technique that has been used to study infants is functional near-infrared spectroscopy (fNIRS). fNIRS measures patterns of the haemodynamic activity based on near-infrared light that is emitted by sources located on the head, is reflected by the cortex, and is detected by the detectors placed in the head. The attenuation (or loss) of this light (in a wavelength range of 650-1000 nm) is due to both absorption and scattering effects within the cortical tissues (Lloyd-Fox, Blasi, & Elwell, 2010). As scattering is assumed to be constant (León-Carrión & León-Domínguez, 2012), the changes measured in the attenuation of near-infrared light can be used for calculate the changes in oxy-haemoglobin (HbO₂), deoxy-

haemoglobin (HHb) and total haemoglobin (HbT) in the eliminated tissue. The changes in the concentration of these chromophores can be used as surrogate markers of brain blood flow and hence provide a means of investigating brain function (Lloyd-Fox et al., 2010); fNIRS is non-invasive, portable and relatively inexpensive with a good spatial resolution (when compared to EEG) and good temporal resolution (higher than fMRI) which makes it a useful technique to study the functional activation of an infant's brain. In recent years, a growing number of studies examined BM processing using fNIRS in infants. Although the number of studies with fNIRS is increasing, most them focused on face perception and used stimulus with intact color and texture information (in contrast to PLWs). Nevertheless, they provided the first important findings: in infants as young as five months of age areas located around the STS region activate to dynamic social stimuli (Lloyd-Fox et al., 2009); infants between seven-eight months show greater differences between HbO₂ and HHb for upright PLD's, compared to inverted PLD and in the right hemisphere (Ichikawa, Kanazawa, Yamaguchi, & Kakigi, 2010).

In a recent study, Lisboa, Miguel, Sampaio, Mouta, Santos & Pereira (2017) conducted an experiment with seven-month infants and fNIRS, using Point-Light Walkers for the first time in a fNIRS study. Participants watched front-facing approaching stimuli in three different experimental stimuli (an intact upright PLW, the same PLW inverted and one single frame of the upright PLW) and their results demonstrated that, in the right middle-posterior temporal cortex, mean concentration change of oxy-haemoglobin was significantly different from baseline only in the upright PLW condition. This was the first study to measure hemodynamic response specifically to an important class of highly-controlled stimuli (PLW's) and the study results supports findings from behavioral studies, only the canonical PLW elicited a haemodynamic response.

In summary, both behavioral and neurophysiological studies indicate that global motion structure is important for BM processing. However, there is no fNIRS study that has directly compared intact global motion structure, as present in canonical PLW's, with a scrambled version of the PLW that maintains the local dot motion but disrupts global motion structure, as present for instance in a PLW where we add a random fixed offset to every point. For this reason, the present study involved a combination of two experimental stimuli: one is the upright PLW used by Lisboa et al. (2017), and the other consist of a spatially scrambled PLW, based on the upright PLW but with a disrupted global motion structure.

Specifically, we ask: in seven-month-olds, the right posterior temporal region has different hemodynamic responses for upright PLW vs a spatially scrambled PLW? We hypothesized that the hemodynamic response is higher for upright PLW compared to a spatially scrambled PLW. This hypothesis is based in accordance with previous behavioral studies that indicate discrimination between PLW vs scrambled PLW's or random motion and ERP's studies that shown higher neural activation for upright PLW when compared with other PLD's.

Methods

Participants

The final sample consisted of eleven infants ($N = 16$, $n = 11$) aged between seven and eight months of age (4 females, mean age = 7 months and 24 days, $SD = 11$ days; see table 1 for details). An additionally five subjects were tested but their data was excluded because they did not accomplish inclusion criteria ($n = 4$) or due to equipment failure ($n = 1$) (attrition rate = 31.25 %). Subjects were recruited through direct contacts with their parents. All parents were informed of the procedure of the experiment and gave informed consent, as approved by the University of Minho.

Table 1

Sample Sociodemographic.

| Characterization of the sample | |
|--------------------------------|--------------------------------|
| Number of infants | 11 |
| Age at birth (weeks) | 39 weeks (12 days) |
| Age at study (Months, days) | 7 months and 24 days (11 days) |
| Weight at birth (g) | 3141.27 (522) |
| Height at birth (cm) | 48.55 (2.52) |
| Cesarean delivers | 1 (9%) |
| Apgar 1 | 9.27 (0.44) |
| Apgar 10 | 10 |
| Head circumference (cm) | 44.78 (1.62) |
| Nasion inion (cm) | 27.24 (1.42) |
| Age of the mother (Years) | 33.27 (3.24) |
| Age of the father (Years) | 35.54 (2.57) |

Note: Standard Deviations appear in parentheses after the mean, except for cesarean delivers that is presented in absolute number followed by the percentage.

A sociodemographic questionnaire was used to evaluate some general information about the infant's development – only typically developing infants were included in the final sample (birth weight > 2kg; gestation period > 37 weeks; Apgar > 7) – see table 1.

Stimuli and Experimental Design

Two different approaching PLWs were used: (1) an Up-Right Point Light Walker (Upright PLW) and (2) a Spatially Scrambled Point-Light Walker (Spatially Scrambled PLW) (see figure 1) both translating over the viewer's frontal plane.

The upright PLW consisted of a male model (13 dots) walking two steps from the back to the front in the frontal plane, for details on motion acquisition and stimulus construction see Mouta, Santos, and López-Moliner (2012); Lisboa et al. (2017). The area covered by the stimulus in visual angle ranged between $13^{\circ} 41'$ x $5^{\circ} 43'$, initially, and $24^{\circ} 48'$ x $24^{\circ} 48'$ at a viewing distance of 50 cm.

For the Spatially Scrambled PLW, each dot of point-light display corresponded to a dot representing a joint of the PLW with exact same motion vectors found in the Upright PLW, but the initial starting positions were randomized. This manipulation left the motion paths of each joint intact, but perturbed the coherent spatial relations among the dots- the global motion structure. Both stimuli were generated with Blender (Open source 3D program), version 2.78, with a resolution of 1440 x 900 pixels and a frame rate of 60 frames.

The stimulus used in the baseline condition consisted of a static image, made from the middle frame, of the Spatially Scrambled PLW; this frame was extracted from another Spatially Scrambled PLW, different from the one used for the experimental condition (see figure 1).

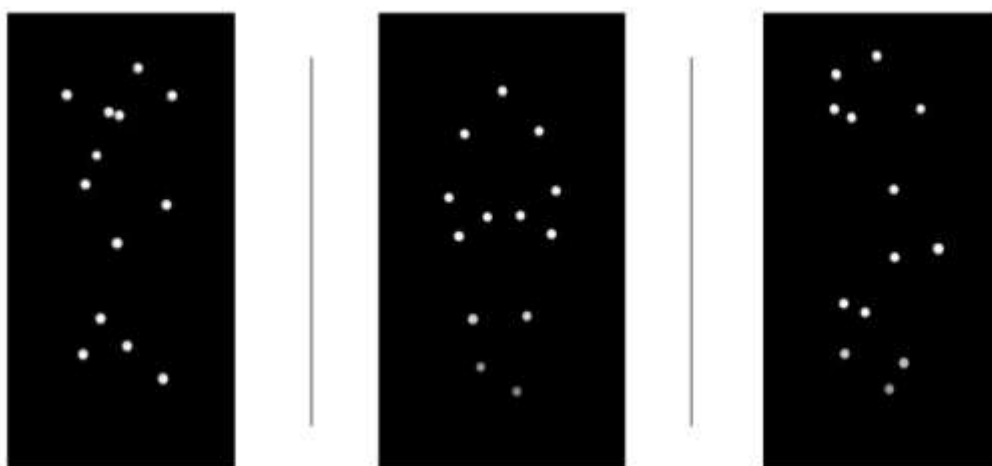


Figure 1. Stimuli used in baseline and experimental conditions (baseline, Up-Right PLW, Spatial Scrambled PLW, respectively).

Subjects participated in a within-subjects blocked design comprised of two experimental conditions (Upright PLW and Spatially Scrambled PLW) and one baseline condition. There were eight blocks: four blocks with an Upright PLW and the other four with a Spatially Scrambled PLW. The presentation order of the blocks was randomized. Each block contained a baseline period (18 seconds) and an experimental period (10 seconds).

In both periods (baseline and experimental), fruits and pleasant sounds were introduced to draw and keep the infant's attention (Ichikawa et al., 2010). Thus, baseline period contained the presentation of a still frame from the Spatially Scrambled SPLW for 4 seconds, followed by the presentation of the fruit image together with the pleasant sound (500 ms) – this was looped four times per block. Additionally, the experimental period comprised the presentation of one of the two experimental stimuli (2 seconds), followed by a fruit with a pleasant sound (500 ms); this sequence was repeated four times per block. The baseline period was longer, in accordance with the majority of studies conducted with infants and fNIRS, to allow the hemodynamic response initiated during the experimental condition to return to a baseline level (Lloyd-Fox et al., 2010), since it is typically necessary more than 10 seconds for the hemodynamic response to return to baseline (Taga, Asakawa, Maki, Konishi, & Koizumi, 2003). Both experimental conditions were randomized and counterbalanced for each participant.

| Baseline | Loop + Fruit | Baseline | Loop + Fruit | Baseline | Loop + Fruit | Baseline | Loop + Fruit | Experimental condition | Loop + Fruit + Blank | Experimental condition | Loop + Fruit + Blank | Experimental condition | Loop + Fruit + Blank | Experimental condition | Loop + Fruit + Blank |
|----------|--------------|----------|--------------|----------|--------------|----------|--------------|------------------------|----------------------|------------------------|----------------------|------------------------|----------------------|------------------------|----------------------|
| 4 s | 0.5 s | 4 s | 0.5 s | 4 s | 0.5 s | 4 s | 0.5 s | 1.5 s | 1 s | 1.5 s | 1 s | 1.5 s | 1 s | 1.5 s | 1 s |

Figure 2. Experimental design.

Procedure

Initially, the parent answered to a sociodemographic questionnaire and measurements of the infant's head circumference, nasion-inion and ear-to-ear distance were made, in order to mark the CZ point in the infant's head. Then, the parent and the infant were sent to the fNIRS room and the probe was placed in infant's head, using the marked Cz as the main reference for cap placement. During the experiment, infants sat on their parent's lap and watched the stimuli on the monitor with a viewing distance of, approximately, 50 cm. The parent was instructed to refrain from interacting with the infant during the stimuli presentation and no talking was allowed during the recording. An experimenter sat next to the infant, tapping the screen to

redirecting his/her attention whenever the infant looked away. Infants were also allowed to use a pacifier or/and rubbers toys to stay calm during the session.

The experiment ended when the infants watched all experimental design (i.e. after 16 trials) or when the infants became bored or fussy. During the experiment, each infant watched the stimulus passively while their brain activity was recorded and their visual fixations were videotaped. Fixation towards the screen was coded to assess the valid trials for each infant. To be included in final sample the following inclusion criteria need to be fulfilled: a) look, at least, five seconds to the experimental stimuli in each trial and b) at least three valid trials in each experimental condition.

To prevent the infant from becoming tired and/or fussy a pause was made on the middle of the stimulus presentation.

NIRS Recording and processing

The UCL optical topography system (Everdell et al., 2005) was used to register the haemodynamic response in the right posterior temporal region. During the experiment, infants used an elastic cap (Easycap, GmbH), that was built using as reference the 10-5 system (Jurcak, Tsuzuki, & Dan, 2007). To ensure the alignment of the probe in the head, with the 10-5 system, we measured head circumference and nasion-inion distance. The mean average for head circumference was 44,78 cm (SD = 1,55 cm) and the nasion-inion distance was 27,24 cm (SD = 1,42 cm). The array was composed of six optodes (that emitted continuous near-infrared light at two fixed wavelengths of 780 and 850 nm) and three detectors (that captured data at 10Hz), containing a total of nine channels (see figure 4). The probe was customized to position these nine channels over the right posterior Superior Temporal area (Lloyd-Fox et al., 2014). The two channels that crossed the middle of array (the longest channels, 12 and 17), had a 54-36mm source-detector separation and the other seven (channels 10, 11, 14, 15, 17, and 18) had 20-22mm. Additionally, we recorded more 9 channels in the motor area but their data was not used in this study. To confirm the correct location of the channels, we took photos of the infant's cap placement.

Raw data was converted into .nirs format for analysis in the HOMER2 system (Huppert, Diamond, Franceschini, & Boas, 2009). The signal in every .nirs files was visually inspected for each channel and channels 12 and 16 were eliminated in all infants because of low intensity of the signal. After, we applied a wavelet motion correction (0.5 Hz) to remove motion artefacts. Following this correction, data was bandpass filtered, using a low-pass filter (0.5 Hz). Then the filtered data of each wavelength was converted to relative concentration of HbO₂ and HHb by applying the Beer-Lambert Law (Kocsis, Herman, & Eke, 2006).

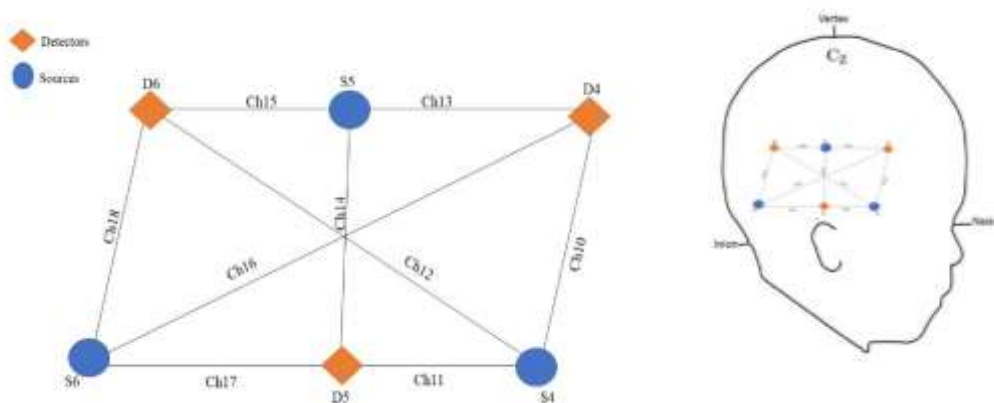


Figure 3. Example of the array and positioning of the probe.

Finally, HbO₂ and HHb concentration was baseline corrected and block averaged from -2 s before the onset of each trial and +10 s after trial offset. The mean concentration changes for each channel was calculated at 10Hz. Based on that, the mean concentration for each channel between all subjects was calculated, for the two experimental conditions.

Results

For statistical analysis, four time windows of 5s after the onset of the stimulus were defined for the mean hemodynamic concentration change analysis. The data was not normally distributed (Shapiro-Wilk, all p 's < .05) and standard transformations to normality did not yield a normally distributed random variable; for this reason, we used a non-parametric test, the Fisher-Pitman Permutation test. Analysis were conducted in R, using the *coin* package (Hothorn, Hornik, Van De Wiel, & Zeileis, 2008).

Initially, statistical tests were conducted, for each channel, on the pre-selected four time windows, and for each condition (Upright PLW and Spatially Scrambled PLW), with the purpose of testing if the concentration changes in HbO₂ were different than zero. After this initial analysis, we identified spatially continuous channels (as in Biondi, Boas, and Wilcox (2016), with similar activation patterns, that could be grouped together and identified one Region of Interest (ROI) defined by channels 10, 11, 13, 14 and 15. The same statistical test was applied in the same time windows, for both experimental conditions. Finally, we tested if the differences in mean HbO₂ between the two experimental conditions were statistically significant.

Individual channel analysis

Fisher-Pitman Permutation test was conducted for HbO₂ and HHb concentration changes and for each channel. Figure 5 shows the average mean concentration changes, HbO₂

and HHb, for all subjects across the six channels (10, 11, 13, 14, 15, 17, 18) and for the two experimental conditions.

Table 2

Number of valid trial for condition for each participant

| Subject | Number of valid trials | |
|---------|------------------------|-------------------------|
| | Upright PLW | Spatially Scrambled PLW |
| 1 | 3 | 6 |
| 2 | 4 | 4 |
| 3 | 5 | 5 |
| 4 | 8 | 7 |
| 5 | 4 | 4 |
| 6 | 6 | 6 |
| 7 | 4 | 7 |
| 8 | 5 | 6 |
| 9 | 6 | 5 |
| 10 | 5 | 5 |
| 11 | 6 | 8 |
| Total | 56 | 63 |

Fisher-Pitman permutation test compared the mean concentration changes in HbO₂ and HHb (during the four time windows previous described) evoked by each experimental condition for the six channels in relation to baseline (see Table 3).

For the Upright PLW condition a significant increase in HbO₂ was found both on channels 10 and 11; marginally significant activations were also found on channel 11 in another time window and on channel 14.

On channel 10, statistically significant results were found around 0-5s ($Z = 1.83, p = .01$) and channel 11 at 10-15s ($Z = 2.05, p = .03$). Additionally, marginally significant results were also found for channel 11 in time window between 15-20s ($Z = 1.72, p = .06$) and for channel 14 between 10-15 ($Z = 1.6, p = 0.09$).

Relative to an HHb decrease, only marginally significant results were found on channels 10 and 15 in the Upright PLW condition. For channel 10 in the time window 15-20s ($Z = -1.74, p = .05$), channel 15 in the time window 10-15s ($Z = -1.75, p = .08$) and 15-20s ($Z = -1.82, p = .06$).

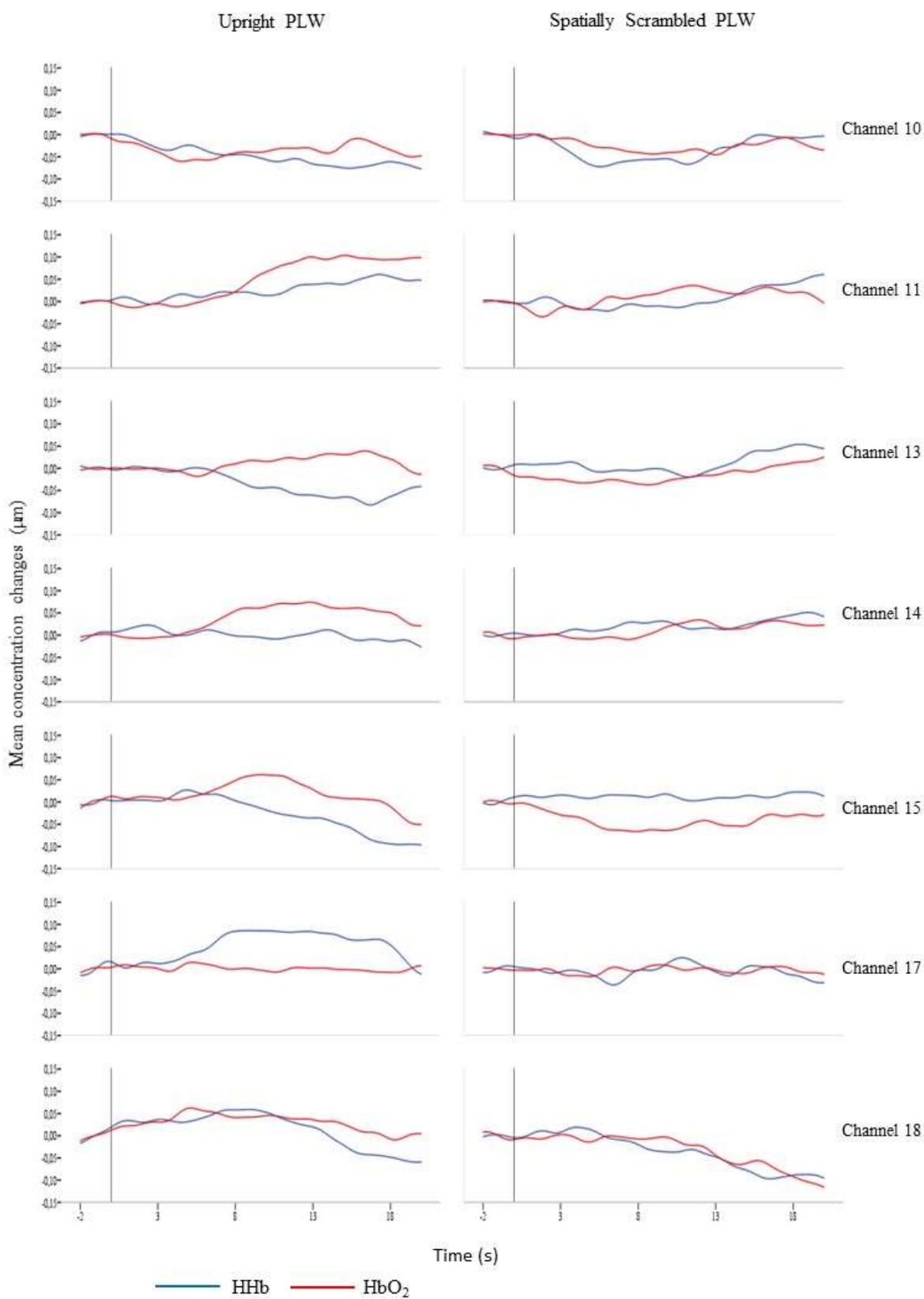


Figure 4: Averaged mean concentration changes(μm) in HbO_2 and HHb across all subjects per channel.

Table 3

Fisher-Pitman Permutation Test results for all channels and Upright PLW and Spatially Scrambled PLW conditions

| Channel | Time window | Upright PLW | | | | Spatially Scrambled PLW | | | |
|---------|-------------|-----------------------------------|--------|---------------------------------|--------|-----------------------------------|--------|---------------------------------|---------|
| | | $\Delta\text{HbO}_2(\mu\text{m})$ | | $\Delta\text{HHb}(\mu\text{m})$ | | $\Delta\text{HbO}_2(\mu\text{m})$ | | $\Delta\text{HHb}(\mu\text{m})$ | |
| | | Z | p | Z | p | Z | p | Z | p |
| 10 |]0,5] | 1.83 | 0.01** | -0.79 | 0.52 | -0.44 | 0.68 | -1.77 | 0.06† |
| |]5,10] | -1.09 | 0.33 | -0.99 | 0.39 | -1.03 | 0.34 | -1.85 | 0.07† |
| |]10,15] | 0.75 | 0.53 | -1.34 | 0.19 | -0.83 | 0.54 | -1.52 | 0.14 |
| |]15,20] | -0.58 | 0.64 | -1.74 | 0.05* | -0.4 | 0.66 | -0.13 | 0.89 |
| 11 |]0,5] | -0.62 | 0.56 | 0.57 | 0.58 | -0.94 | 0.38 | -0.28 | 0.82 |
| |]5,10] | 0.85 | 0.43 | 0.61 | 0.56 | 0.31 | 0.79 | -0.88 | 0.43 |
| |]10,15] | 2.05 | 0.03* | 0.78 | 0.5 | 0.54 | 0.64 | -0.00 | 1 |
| |]15,20] | 1.72 | 0.06† | 0.89 | 0.5 | 0.43 | 0.67 | -0.95 | 0.39 |
| 13 |]0,5] | -0.15 | 0.9 | -0.12 | 0.93 | -1.59 | 0.11 | 0.58 | 0.56 |
| |]5,10] | 0.13 | 0.93 | -0.79 | 0.51 | -1.54 | 0.15 | -0.12 | 0.91 |
| |]10,15] | 0.94 | 0.36 | -1.58 | 0.13 | -0.63 | 0.55 | -0.05 | 0.98 |
| |]15,20] | 0.46 | 0.67 | -1.09 | 0.33 | 0.3 | 0.78 | 1.30 | 0,24 |
| 14 |]0,5] | -0.23 | 0.82 | 0.63 | 0.72 | -0.29 | 0.78 | 0.37 | 0.73 |
| |]5,10] | 1.44 | 0.17 | 0.05 | 0.95 | -0.08 | 0.94 | 0.97 | 0.44 |
| |]10,15] | 1.6 | 0.09† | 0.02 | 0.98 | 1.24 | 0.24 | 0.77 | 0.46 |
| |]15,20] | 0.82 | 0.63 | -0.19 | 0.86 | 0.99 | 0.36 | 1.06 | 0.30 |
| 15 |]0,5] | 0.32 | 0.94 | 0.37 | 0.70 | -1.65 | 0.08 | 0.74 | 0.51 |
| |]5,10] | 1.29 | 0.21 | 0.22 | 0.85 | -2.33 | 0.01** | 0.45 | 0.74 |
| |]10,15] | 1.23 | 0.22 | -1.75 | 0.08† | -2.02 | 0.03* | 0.24 | 0.86 |
| |]15,20] | -0.38 | 0.73 | -1.82 | 0.06† | -0.93 | 0.41 | 0.29 | 0.8 |
| 17 |]0,5] | 0.14 | 0.92 | 1.10 | 0.28 | -0.49 | 0.65 | -0.36 | 0.72 |
| |]5,10] | 0.06 | 0.96 | 1.94 | 0.04* | 0.00 | 0.99 | -0.42 | 0.68 |
| |]10,15] | -0.08 | 0.99 | 1.62 | 0.01** | -0.26 | 0.8 | 0.08 | 0.94 |
| |]15,20] | -0.05 | 0.98 | 0.68 | 0.55 | -0.08 | 0.95 | -0.17 | 0.87 |
| 18 |]0,5] | 0.99 | 0.35 | 1.08 | 0.34 | -0.29 | 0.81 | 0.84 | 0.44 |
| |]5,10] | 0.89 | 0.41 | 1.28 | 0.23 | -0.19 | 0.86 | 0.73 | 0.48 |
| |]10,15] | 0.71 | 0.49 | 0.45 | 0.67 | -1.53 | 0.09 | -2.46 | 0.01** |
| |]15,20] | 0.09 | 0.92 | -0.94 | 0.35 | -1.85 | 0.05* | -2.73 | 0.00*** |

Note: † $p \leq .1$; * $p \leq .05$; ** $p \leq .01$; *** $p \leq .001$

For the Spatially Scrambled PLW condition, a significantly decrease in HbO₂ changes in the channel 15 for time window between 5-10s ($Z = -2.33, p < .01$) and 10-15 s ($Z = -2.02; p = .03$) was registered. Also, marginally significant decreases were registered for channel 15 in time window between 0-5s ($Z = -1.65, p = .08$) and for channel 18 in the time window 10-15s ($Z = -1.53, p = .09$) and 15-20s ($Z = -1.85, p = .05$). Importantly, these results are not associated with a typical NIRS' functional response.

On the contrary, HHb significant changes were associated with a functional response on channel 18, that showed a significant HHb decrease between 10-15s ($Z = -2.46, p = .01$) and 25-20s ($Z = -2.73, p < .01$). Channel 15 also registered marginally significant decreases in HHb changes in time window 0-5s ($Z = -1.77, p = .06$) and 5-10s ($Z = -1.85; p = .07$).

ROI analysis

Fisher-Pitman Permutation test results are summarized in table 4. Marginally significant increases in HbO₂ changes were found for Upright PLW, in the time window 10-15s ($Z = 1.84, p = .06$). For Spatially Scrambled PLW we did find any significant increase in HbO₂ changes.

Finally, both experimental conditions (Upright PLW and Spatially Scrambled PLW) were tested (see figure 6) for a mean concentration changes in HbO₂, in the time window 10-15s. The Fisher-Pitman Permutation test result indicated a significant difference between the conditions 10-15s ($Z = 2.05, p < 0.05$).

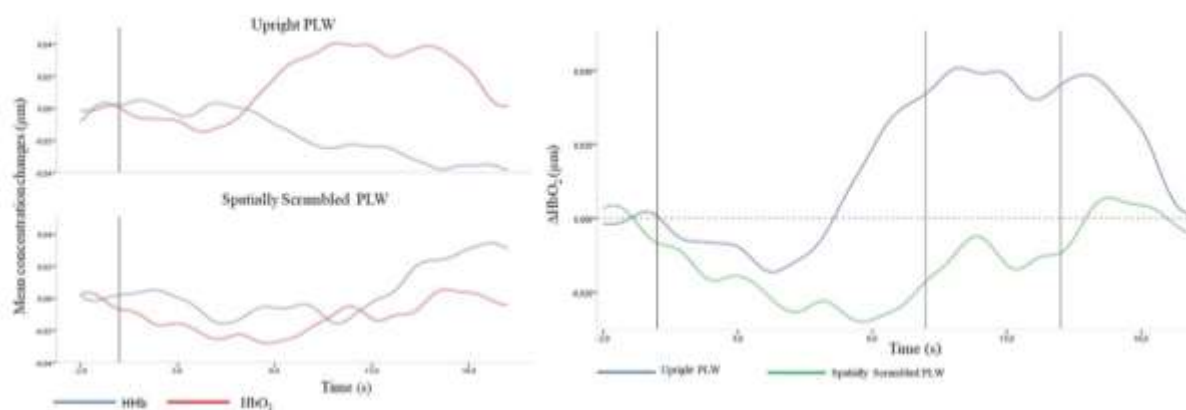


Figure 5. Mean concentration changes, in ROI, for Upright PLW and Spatially Scrambled PLW (left). HbO₂ mean concentration changes for Upright PLW and Spatially Scrambled PLW, in ROI (right).

Table 4

Results of Fisher-Pitman Permutation test for ROI analysis

| Condition | Time Window | ΔHbO_2 (μm) | | ΔHHb (μm) | |
|-------------------------|-------------|--|-------------------|--------------------------------------|----------|
| | | Z | <i>p</i> | Z | <i>p</i> |
| Upright PLW |]0,5] | -0.79 | 0.45 | 0.09 | 0.92 |
| |]5,10] | 0.93 | 0.37 | -0.53 | 0.64 |
| |]10,15] | 1.84 | 0.06 [†] | -1.14 | 0.28 |
| |]15,20] | 0.74 | 0.51 | -0.88 | 0.45 |
| Spatially Scrambled PLW |]0,5] | -1.11 | 0.29 | -0.59 | 0.93 |
| |]5,10] | -1.13 | 0.30 | -0.39 | 0.72 |
| |]10,15] | -0.41 | 0.69 | -0.21 | 0.87 |
| |]15,20] | 0.025 | 0.98 | 1.09 | 0.30 |

Note: [†] $p \leq .1$

Discussion

We measured the response in the right posterior temporal region to Upright PLW and Spatially Scrambled PLW and hypothesized that an Upright PLW would elicit higher neural activations, as measured by the increase in HbO₂ concentration, compared to a Spatially Scrambled PLW. Our fNIRS array and the use of this technique does not allow for a direct localization in the right STS – the array measured a larger right posterior temporal region; we can infer that the area covered for the array is the pSTS because of a co-registration study, between fNIRS and fMRI, conducted by Lloyd-Fox et al. (2014). The fNIRS response, for Upright PLW condition, indicates significant increases in HbO₂ in two individual channels (channel 10 and channel 11) and a marginally significant increase in channel 14. Additionally, when the signal was visually inspected (see figure 4) the typical functional fNIRS response – increase in HbO₂ and decrease in HHb – could be observed in all channels, except for channels 17 and 18 in the Upright condition. In contrast, in the Spatially Scrambled PLW condition, increase in HbO₂ was not significant in any of the individual channels and the visual inspection of the hemodynamic response does not reveal the typical functional fNIRS activation.

Typical functional response in fNIRS in adults shows an increase in oxy-haemoglobin (HbO₂) and a (relatively smaller) decreased in deoxy-haemoglobin (HHb) (Villringer & Chance, 1997). However, this pattern of response is not so clear in infants; (Lloyd-Fox et al., 2010) conducted a review across studies done with infants and fNIRS and concluded that only

HbO₂ changes related to cortical activation were highly consistent across studies and also similar to what is observed in adults. Usually, HHb changes are far less consistent and often are not reported (Biondi et al., 2016).

The results of the ROI analysis revealed the typical pattern of functional fNIRS response for the Up-Right PLW but not for the Spatially Scrambled PLW condition. Moreover, the increase in HbO₂ was statistically significant for the Upright PLW in the time window 10-15s.

These results agree with our research hypothesis and with previous research, i.e., the right pSTS have a certain specialization for processing BM and that ability is present in early infancy. The pattern of activation in right pSTS seems to be localized in an area of STS, specifically in the posterior area to exactly (ROI). Furthermore, these results also replicate activations reported by Lisboa et. al (2017) with inverted and rigid PLWs. They are also in accordance with previous behavioral data and previous EEG and fNIRS experiments.

Behavioral studies showed that infants discriminate canonical PLWs from similar displays with a spatially scrambled relation (Bertenthal, Proffitt, & Kramer, 1987) or with random motion (Fox & McDaniel, 1982). Previous studies conducted with infants and the PLD technique, using different neuroimaging techniques, also found higher neural activations for canonical PLW when compared to scrambled motion (Hirai & Hiraki, 2005) and inverted PLW using EEG (Reid et al., 2006). The results of another experiment conducted with EEG, PLD technique and five month old infants (Marshall & Shipley, 2009) show that disruptions in global form are critical for the processing of upright human motion. Most studies conducted with fNIRS with the aim of study the BM's processing usually do not use PLD technique but full-color videos. For that reason, all comparisons that can be made are limited for that difference. However, we speculate, based in our results and previous studies, that by seven-months-olds, infants already began to mature the neural circuits responsible for BM perception since that we found neural activation in right pSTS, the same area activated for BM in adults. The global structure of the stimuli also plays an important role for that process, infants show fNIRS functional response for Upright PLW but not for Spatially Scrambled PLW, consequently human form seems critical for BM's perception. But this raises an important question: how and when do human infants develop a meaning for human figure? The area activated, right pSTS, is also linked to social cognition what seems indicate that BM perception and social cognition are related since very early, as has been reported in the literature. The findings of this study become more interesting because we observed activations even with minimal information, one characteristic of PLD technique. This seems indicated that our ability of processing global form of BM could play an important role for our social cognition. Another important question raised

by our results is: is it possible that subjects with impairments in BM processing show a deficit in social development? The research field of the link between BM and social cognition is relatively new but clearly is a research field that need more studies.

In the present study, the stimuli used maintained the translation component, which is an innovation since most studies conducted in this research field use stimuli without translation (e. g. treadmill). The problem of using stimuli without translation is the lack of an ecological approach since that the movement of others human beings that we perceive in our everyday life is composed by that component. The importance of the translation displacement in BM' s perception has become an important topic of discussion. A study conducted by Bidet-Ildei et al. (2014), with newborns, suggested that translational displacement is important for visual processing of BM. When newborns were presented with a PLW and random motion, both with translation, did not exhibit any preference. However, our results, with an Upright PLW and Spatially Scrambled PLW, show a different result for infants with seven-months-old. Although both stimuli have translational displacement was exhibit higher neural response to Upright PLW condition. In this sense, it will be interesting conducted the same experimental design of this study with newborns to verified if the pattern of the haemodynamic response is different from what was found in this study.

The difference registered between the channels of ROI and channels 17 and 18 could be an interesting result, some previous studies (Lloyd-Fox et al., 2009; Pelphrey, Morris, Michelich, Allison, & McCarthy, 2005) already report similar results, however need complementary studies.

This study was also a continuation of the study conducted by Lisboa et al. (2007) with some methodological changes, especially in the duration of the stimuli. Our results indicate that even with shorter stimuli it is possible to elicit the typical hemodynamic response. This is an important experimental improvement given that has reduced the attrition rate. Another difference between both experimental designs was the number of trials. The experimental designed of Lisboa et al. (2017) comprised 48 trials and the one used in this study comprised 32trials. The reduction of trials also was important for the reduction of the attrition rate.

In summary, this study seems be in accordance with previous research about the neural basis of BM's processing. The results indicate that by seven-months old infants already activate the same brain area, right pSTS, that adults for BM. The form also seems be an important feature for this activation, what is in accordance with previous EEG studies. As a continuity study of Lisboa et al. (2007), our results corroborate the previous findings and the new experimental design adjustments revealed themselves as an important improvement.

Limitations

This study had some limitations. The main limitation was the small sample. The haemodynamic response in infants have a significant variance and as our sample was small this variance had some implication in results.

Another limitation of this study is related with the material. The array used only allow recording for the right pSTS, but it would also be interesting to record the left pSTS, in order to understand if the pattern of haemodynamic response is the same. It has been shown that the processing of BM's seems to be lateralized to the right hemisphere in adults but can we observe the same at seven months old?

The combination between fNIRS and fMRI would be interesting for a better understanding of the hemodynamic response in pSTS in infants. fNIRS is considered a good technique but the spatial resolution of data does not allow answer some question raised, as the existence of a specific area in right pSTS for BM.

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