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Effective connectivity analysis of brain networks in preterm infants

A Master Thesis

By

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The undersigned have examined the thesis entitled '**Effective connectivity analysis of brain networks in preterm infants'** presented by **Serafeim LOUKAS**, a candidate for the degree of **Master of Science (Master in Neuroscience)** and hereby certify that it is worthy of acceptance.

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ABSTRACT

Preterm birth is the most common cause of death among infants worldwide. The percentage of preterm births has shown a steady increase over the last years. The chance of survival is increasing by every gestational week and reaches almost 100% at 31-32 weeks. The cause of preterm birth is often not known. A variety of risk factors has been established including maternal infection and inflammation, diabetes, obesity or maternal underweight, high blood pressure, smoking and high stress. After birth, preterm infants need immediate care and support. Neonatal Intensive Care Units (NICU) serve this goal by providing special equipment designed to give life support and protect these preterm newborns. In recent years NICU care has been modified to include developmentally oriented care. In this context, Music has been introduced into the NICU as an environmental modulator that may enhance treatment and facilitate growth and development of premature infants. Music in other settings has been shown to have significant benefits across a variety of physiological and behavioral measures. In this study we examined the impact of the environmental enrichment of preterm newborns with music on auditory cortex functional connectivity. A group of preterm infants listened to music from 33 weeks gestational age until term equivalent age. Two control groups were used: preterm and full-term infants without music. Auditory cortex functional connectivity with cerebral regions known to be implicated in tempo and familiarity processing were identified for preterm newborns that had music training during their stay in the NICU using Psychophysiological Interaction (PPI) analyses. Our

results suggest that NICU's environment can induce brain functional connectivity changes that are associated with learning. Thus, the NICU environmental stimuli need to be carefully and wisely chosen in order to be beneficial for the newborns.

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CHAPTER I: Introduction

Early brain development

The important health consequences for preterm birth and perinatal care stretch from the neonatal period to childhood and adulthood, with its effects ranging from motor disabilities to difficulties in cognitive domains such as attention, memory, reading, mathematics, to reasoning and emotion regulation (Larroque et al., 2011; Kwon et al., 2014). The basis of these cognitive and behavioral consequences is related to the alteration of early brain development.

Development of cerebral cortex is a complex process of well-ordered spatio-temporal events that could be grouped in three principal periods: layer formation, pre-wiring and activity-dependent plasticity (Kiss et al., 2014). Representing execution of the intrinsic developmental program the first two periods mainly finish before birth and are highly genetically controlled while the period of activity-dependent plasticity starts with the appearance of extrinsic stimuli necessary to finalize cortical maturation. The fact that the developing cortex of preterm infants is exposed to the extrinsic stimuli before time may lead to an early switch to the activity-dependent network establishment before ending of the pre-wiring period.

The third trimester of gestation is crucial for pre-wiring neuronal networks organization, myelination of axons, synaptogenesis, cortical folding and brain growth (Dubois et al., 2008; Kostovic & Vasung 2009), explaining the high vulnerability of preterm infants to altered brain development.

Auditory system development

The sensory systems become functional in a specific and invariant sequence across early development: tactile>vestibular>chemical>auditory>visual. The development of the auditory cortex is a complex process that begins early in gestation. Structural parts of the ear, including the cochlea, develop early: by 20 weeks of gestation auditory system is anatomically formed but it becomes functional at around 26-30 weeks of gestation (Ruben et al., 1995) when the thalamo-cortical connections with the auditory cortex are established (Kostovic & Vasung 2009). The period between 26 and 30 weeks of gestation is the time when the hair cells in the cochlea are fine-tuned and able to translate acoustic stimuli (vibrations) into electrical signals that are transferred to the brain (Querleu et al., 1989). After 30 weeks of gestation, the auditory system, the axons of the auditory nerve, and the neurons of the temporal lobes' auditory cortex are capable of distinguishing between different speech phonemes (Cheour-Luhtanen et al., 1996; Hepper et al., 1993), which supports early development of language and speech (Mehler et al., 1988).

Music and Sound processing in infants

Over the last decade, studies have further been aimed at understanding how newborn infants process sounds and music, defining laterality and cortical response. Eldredge and Salamy (Eldredge & Salamy 1996) showed that there is right ear advantage in the processing of auditory signals. In this study they used bilateral auditory evoked brain responses (different latencies) (ABR recordings in 452 infants 32-45 weeks postconceptional age. Perani et al., using fMRI in 1 to 3 day old newborns proposed that there is a right hemispheric dominance for music processing. Interestingly, they also showed that neural architecture underlying music processing is sensitive to changes in tonal key (Perani et al., 2010). Additionally, it has been shown, using event related potentials ERPs (and in particular with mismatch negativity) that newborns can discriminate between dissonant and consonant and between major and minor chords (Virtala et al., 2013). Kotilahti (Kotilahti et al., 2010) on the other hand, using near-infrared spectroscopy (NIRS) on the auditory cortices, did not show any lateralization in music processing in newborns.

Tempo and beat processing in infants

In a study using event related potentials, Full-term newborn infants were shown to be able to detect the temporal structure of sound sequences. In this study, significant event-related potentials were induced by onsets and offsets of tone trains as well as changes in tempo (presentation rate) (Haden et al., 2015). Another study using ERPs again showed that newborn infants were anticipating the onset of rhythmic cycles suggesting that beat perception is innate (Winkler et al., 2009). Additionally, a predisposition for rhythmic movement has been proven to be present in response to music in infants from 5 to 24 months (Zentner & Eerola 2010). Finally, Volkova et al. reported that seven-month-olds infants reacted differently for different pitch levels (longer listening to novel pitch level) (Volkova et al., 2006).

Learning capacity in infants and fetuses

Interestingly, fetuses and full-term infants have been shown to react to familiar sounds such as their mother's voice, music, phonemes etc. (Hepper et al., 1993). Indeed, fetuses exhibited changes in their movements when played a tune heard previously during pregnancy (Hepper 1991). Furthermore, fetal auditory learning has been claimed in newborns that were listening to the same music they had heard during the last trimester of gestation, which elicited enhanced brain activity (mismatch negativity, ERPs) in response to pitch changes for the trained variants after birth (Partanen et al., 2013). Additionally, after one week of exposure 6 month old infants seemed to remember the melody, but also the tempo and timbre information of the music they heard (head-turn preference procedure) (Trainor et al., 2004). Another study claimed that 2 to 4 month old infants could discriminate music that they were used to and music played 15% faster, based on observations of infants gaze direction (Baruch & Drake 1997).

Critical period of auditory system

Premature newborn infants spend their first weeks of life in the Neonatal Intensive Care Unit (NICU). NICU provides support and care for these vulnerable infants. However, during this period they are on one hand deprived of the biological maternal sounds that they would be hearing in utero (McMahon et al., 2012) and on the other hand exposed to various external sounds of mechanical as well as human origin. This change in stimulation when the auditory system is at a critical period for development can lead to altered maturation of the auditory brain (Neville & Bavelier 2002) resulting in speech and language acquisition deficits (Fifer & Moon 1994; Shahidullah & Hepper 1994). Importantly, the development of the auditory cortex is dependent on the acoustic environment as demonstrated by animal studies. For instance, auditory deprivation has been shown to decrease the NMDA receptor expression levels in the rat auditory cortex during the early postnatal development period (Lu et al., 2008; Bi et al., 2006). Additionally, the deprivation of auditory experience in young birds has been proven to delay the onset of topographic brain organization (Iyengar & Bottjer 2002). Furthermore, infant rats raided under sensory deafness conditions, developed abnormal synaptic morphology in the primary auditory cortex (Bose et al., 2010). To overcome and address the problem of auditory deprivation, auditory meaningful sounds (like voice and music in the neonatal intensive care unit (NICU)) may be a helpful adjunct for the development of the tonotopic columns in the auditory cortex and for the critical tuning of the hair cells in cochlea that normally occurs during the third trimester of gestation.

Noise in NICU and its effects

The second major problem that needs to be addressed is the noise. Undoubtedly, the NICU's environment is contaminated with noise coming from monitors, pagers, ventilators as well as alarms (Graven 2000). Over the last decade, more and more studies have examined the negative effects of noise on preterm infants in the NICU. It has been proven that noise can induce changes in heart rate (Field et al., 1979), blood pressure (Vranekovic et al., 1974), respiration and oxygenation (Wharrad & Davis 1997). Additionally, noise causes increased crying and reduced sleep (Strauch et al., 1993) and can negatively affect the cardiovascular and respiratory systems resulting in developmental deficits (Wachman & Lahav 2011). Curiously, exposure to noise during pregnancy caused growth retardation, decreased neurogenesis in the hippocampus and impaired spatial learning ability in rat pups, whereas exposure to music increased neurogenesis in the hippocampus and enhanced spatial learning ability (Kim et al., 2006). Furthermore, background noise level greater than 60 dB interferes with recognition and frequency discrimination in preterm infants. (Graven & Browne 2008; McMahon et al., 2012; Chaudhury et al., 2016). Taken all these together, we could argue that it is very important to address the noise problem by improving the NICU design, modifying the equipment and measuring the noise levels frequently.

Acoustic Enrichment of NICU's environment

As mentioned before it is very important to address the problem of auditory deprivation by enriching the NICU's environment. For this reason, music therapy has been proposed in the NICU. Unlike noise that is a discordant combination of frequencies, music is composed of recognizable patterns of changes in wavelength and amplitude that can positively influence physiological and behavioral state. Prenatal music exposure in chicks has been shown to increase volume and neuronal number of the auditory thalamus nucleus ovoidalis (Kumar & Wadhwa 2014). Acoustical enrichment during early postnatal period in rats seems to have long-lasting positive impact on brain development (Kim et al. 2006, Chikahisa et al. 2006). Kim et al (Kim et al., 2013) showed in a recent study that music exposure during the fetal period increased the neurogenesis in the motor and somatosensory cortex in rat pups. In contrast, rat pups exposed to noise during pregnancy showed decreased neurogenesis and thickness in the motor and somatosensory cortex. Furthermore, contrary to noise exposure, music in the perinatal period of mice enhances learning performance in adults by activating neurotrophic pathways in the brain (Chikahisa et al. 2006). Early auditory enrichment with music has also been shown to enhance learning ability in auditory signal detection task and in sound duration-discrimination task, by influencing NMDA-mediated neural plasticity (Xu, Yu et al. 2009). Taken all these together, we could argue that acoustic enrichment of the NICU'S environment, has positive effects.

Effect of music on preterm infants

In preterm infants, music has been shown to slow down heart and respiratory rate (Collins & Kuck 1991; Keith et al., 2009), improve oxygen saturation (Chou et al., 2003), and reduce energy expenditure as well as the duration of hospitalization (Standley 2012), and to mature the sleep wake cycle on EEG (Haslbeck 2012; Burke et al., 1995). Furthermore music processing, neural emotional responses to musical stimuli and sensitivity of the auditory cortex to consonance and dissonance are already present in three-days-old full-term infants (Perani et al., 2010; Virtala et al, 2013). Preterm infants with auditory enrichment of their environment using womb-like maternal sounds during one month presented larger auditory cortex (Webb et al., 2015). Finally, 2 months old infants increased the rhythm of non-nutritive sucking when external tempo was 15% faster than neutral sucking rhythm (Bobin-Begue et al., 2006).

Thus, music intervention in NICU, as early enrichment of preterm environment might have an impact on preterm brain development and maturation. Despite a large literature in basic science on environmental enrichment, to date few studies have addressed the role of music in the enhancement of environment for the premature infant and its impact on brain functional connectivity. In the current study a group of preterm infants were listening to short pieces of specifically composed music during their NICU stay and compared to a control group of preterm infants.

Hypothesis of the current study: Music in preterm infants changes functional brain connectivity

The present study aims to explore the functional connectivity of the primary auditory cortices (seed regions) with the entire brain and how it is modulated by the different types of musical conditions (Original music, Tempo modification, Key modulation, Background music) using Psychophysiological Interaction (PPI) analysis on preterm infants at term and full term infants.

A group of preterm infants had listened to the Original music for several weeks during their NICU stay prior to term age and another group of preterm infants received a caregiver who was blinded intervention with headphones but without music (more details in Methods chapter).

CHAPTER II: METHODOLOGY

Subjects

Three groups of newborns were recruited. The first group (Music group) consisted of eighteen preterm newborns (Mean Gestational Age (GA): 28.83 weeks ± 2.14), which were listening to music with headphones five times per week during the hospitalization in the NICU. The second group (Non-music group) consisted of seventeen preterm newborns (Mean Gestational Age (GA): 29.11 weeks ± 1.85), which had headphones without music five times per week during the hospitalization.

A magnetic resonance imaging (MRI) scan was conducted as soon as the Music and Non-music preterm newborns reached the term-equivalent age (Gestational Age (GA) at scan: music group: 40.35 weeks ± 0.73; non-music group: 40.5 weeks ± 0.77). The third group (Term group) consisted of twenty-one full term newborns (Gestational Age (GA): 39.44 weeks ± 1.12) scanned in the first days of life (Gestational Age (GA) at scan: 39.73 weeks ± 1.07).

The Music group listened to a music especially created by Andreas Vollenweider, composed of a soothing background sound (voices, harp), and bells, harp and punji (charming snake flute) from 33 weeks gestational age until the MRI (mean number of listening: 25.22 ± 9.78). This music was composed after testing for behavioral responses evaluated by a nurse specialized in developmental care.

The Non-music group had the headphones put on without music (mean number of time: 25.89 ± 5.64) during the same period in order to blind the care team.

MRI Data Acquisition

Siemens 3T scanners (Siemens Trio: 11 music-infants, 9 non-music infants, 15 full-term infants; Siemens Prisma: 7 music-infants, 8 non-music infants, 6 full-term infants) were used to obtain T2*- weighted gradient-echo EPI images (TR = 1600 ms, TE = 30 ms, 30 slices, voxel size= 2.5 x 2.5 x 3.0 mm^3) as well as T2 - weighted TSE image for anatomical reference (TR = 4600ms, TE =150ms, 113 coronal slices, voxel size =0.78 x 0.78 x 1.2mm^3).

MRI Experimental Design

During the EPI sequence, music stimuli were presented in a pseudo-random block design protocol. The duration of each block was 8 seconds (5 conditions: Silence, Background Music, Original Music (instrumental music), Transposed Music (key modulation B/Eb of the original music), and Tempo Music (original music played 40% faster). Each stimulus was repeated ten times. Additionally, resting-state functional MRI data as well as structural images were acquired before and after the presentation of the music stimuli (fMRI Music) for further analysis (**Figure 1**).

All infants were fed before the MRI and swaddled in a blanket. No sedation was used, infants were scanned while laying quietly in the scanner or sleeping. To protect infants from the noise of the scanner and to deliver the music, MR-compatible headphones have been used (MR confon, Magdeburg, Germany).

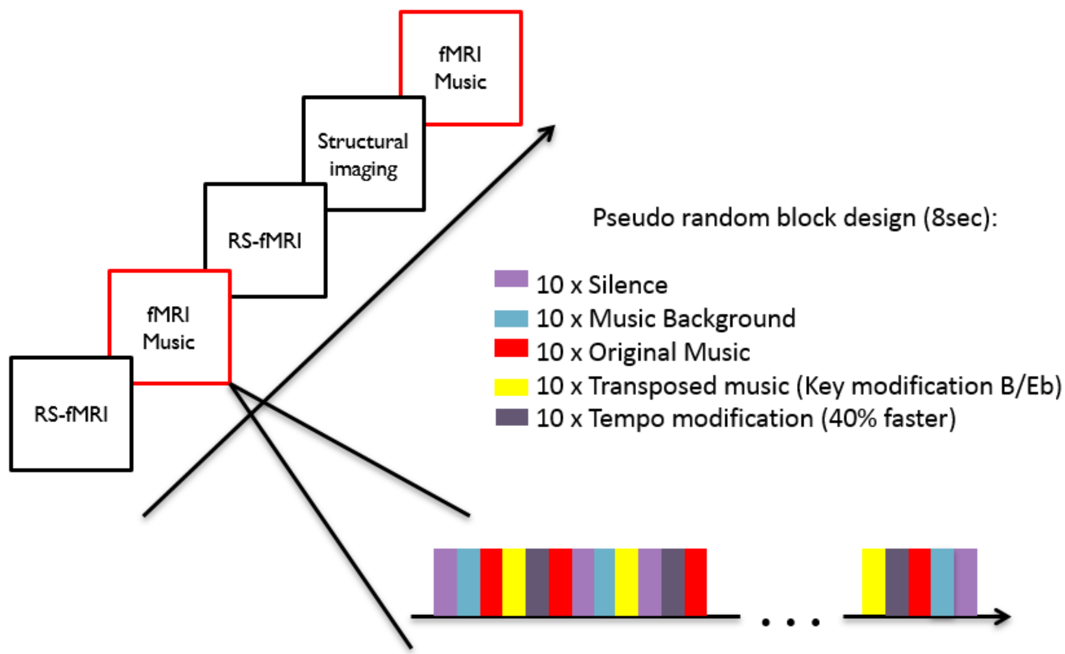

Figure 1: The experimental design of the study.

Preprocessing

Functional MRI sequences were preprocessed and analyzed using Statistical Parametric Mapping software SPM8 [Wellcome Department of Imaging Neuroscience, London (www.fil.ion.ucl.ac.uk/spm/software/spm8/)] including: (i) realignment; (ii) slice-timing; (iii) rigid body co-registration with the T2 structural image; (iv) normalization of the T2 structural image (1 x 1 x 1 mm^3) and the EPI (2 x 2 x 2 mm^3) using a newborn template (N = 20 term and preterm at TEA newborns); and (v) smoothing with a Gaussian kernel of 6-mm full-width at half-maximum.

To correct for motion, 24 motion-related parameters (the 6 realignment parameters with a Volterra expansion) were also included in the model as covariates to remove any residual motion-related variance (Friston et al., 1996).

Exclusion Criteria

Only infants with absolute motion under 1 mm for all images or for more than 180 consecutive images (70% of the total run) where used for subsequent analyses. Additionally, all subsequent analyses were performed only on infants showing activations induced by the overall sound versus silence contrast, at $p < 0.01$ uncorrected in auditory regions. Infants were excluded because of major brain lesions, large-scale movement or because no auditory activation to sound has been found. Finally, infants with brain lesions were excluded resulting in 9 music-infants (mean GA at birth: 28.7 ± 2.46 weeks, mean GA at MRI: 40.25 ± 0.51 weeks), 9 no-music infants (mean GA at birth: 28.7 ± 2.01 weeks, mean GA. at MRI: 40.4 ± 0.77 weeks) and 9 full-term infants (mean GA at birth: 39.32 ± 1.03 weeks, mean GA at MRI: 39.63 ± 1.02 weeks).

Data Analysis

In order to investigate if the music intervention during the NICU stay has an impact on preterm brain functional connectivity, Psychophysiological Interaction (PPI) analyses were conducted. PPI analysis is a model-based approach that allows assessing effective connectivity. The term effective connectivity refers to the influence that one neuronal system exerts over another (Friston et al., 1997).

A PPI analysis investigates task-specific changes in the relationship between activity in different brain areas (Friston et al., 1997) and aims to indicate which voxels in the brain increase their relationship with a seed region of interest in a given context, such as during a specific behavioral task (in this case during listening to music) (O'Reilly et al., 2012). A task-specific increase in the relationship between brain regions suggests a task-specific increase in the exchange of information. In other words, a PPI analysis aims to explain neural responses in one brain area in terms of the interaction between influences of another brain region and a sensory/cognitive process.

The PPI design matrix consists of three main regressors (aside from confounds like motion regressors and the baseline) (**Figure 2**): (i) the ‘’psychological variable’’ or “task time course” which is a vector coding for a specific task (e.g., 1 for Original Music and -1 for Tempo modification) convolved with the hemodynamic response function, (ii) the interaction term which is the element-by-element product of the psychological and physiological factor and (iii) the ‘’physiological variable’’ which is the BOLD time course of a specific seed region (in this study the left and right primary auditory cortex).

The equation that describes the PPI analysis is given by Eq. (1).

$$\boldsymbol{y} = \boldsymbol{T} * \boldsymbol{\beta_1} + \boldsymbol{S} * \boldsymbol{\beta_2} + (\boldsymbol{T}\ \boldsymbol{x}\ \boldsymbol{S}) * \boldsymbol{\beta_3} + \boldsymbol{G} * \boldsymbol{\beta_4} + \boldsymbol{\varepsilon} \qquad (\mathbf{1})$$

where Y is the measured BOLD signal at each voxel in the brain, T is the main task effect (third column in PPI design matrix), S is the main effect of condition (first column in the PPI design matrix), T x S is the interaction term (second column in PPI design matrix), G are the confounds such as movement parameters, ε is the error term and β are the parameter estimates. The inclusion of main effects, when performing a PPI analysis and estimating interactions, is important since without them we would not be sure that estimates of the interaction term are not confounded by main effects (Friston et al., 1997).

A PPI analysis involves the following steps: (i) perform a standard General Linear Model (GLM) analysis; (ii) extract the blood-oxygenation-level dependent (BOLD) signal from a seed region of interest identified in the GLM analysis; (iii) form the interaction term; and (iv) perform a second GLM analysis that includes the interaction term, the physiological variable and the psychological variable (aside from confounds and error regressors) in the (PPI) design matrix.

In detail, at the first level, a standard GLM analysis was conducted for each subject. The four experimental conditions (Original music, Key modulation, Tempo modification and Background music) as well as the baseline condition (no music, silence) were included in the design matrix. Task regressors contained the onsets of the blocks of each condition, respectively. For each session, the four conditions were modeled as a boxcar function convolved with the canonical hemodynamic response function (HRF).

After defining the contrasts of interest (e.g., Original music vs Tempo Modification, Original music vs Key modulation etc.), BOLD signals were then extracted from each seed regions of interest (Left and Right Primary Auditory cortex) for each subject. Then two standard psychophysiological (PPI) analyses were carried out (one for each seed region respectively) for each subject (**Figure 3**). For the PPI analyses the gPPI toolbox, which also supports standard PPI analysis, was used (McLaren et al., 2012).

Finally, subject-specific contrast images using the contrast (0 1 0), where the second column represented the psychophysiological interaction (PPI) term (**Figure 2**), were then entered into second level analyses (corrected for multiple comparisons at cluster level with a voxel-level threshold of $p < 0.005$ uncorrected and extent threshold 20 voxels in all cases) to identify clusters of voxels for which the psychophysiological interaction effect was significant.

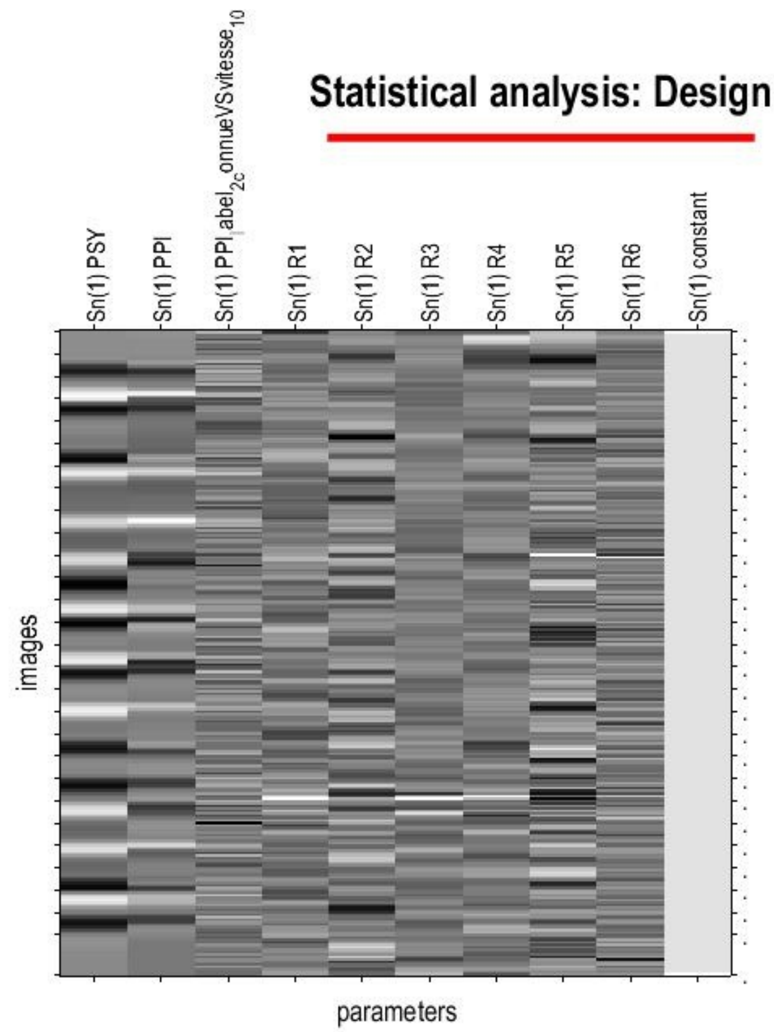

Figure 2: The PPI design matrix: the first column represents the psychological variable (task time course), the second column represents the interaction term and the third column is the BOLD signal from the seed ROI. The next six columns are the motion regressors and the last is the baseline.

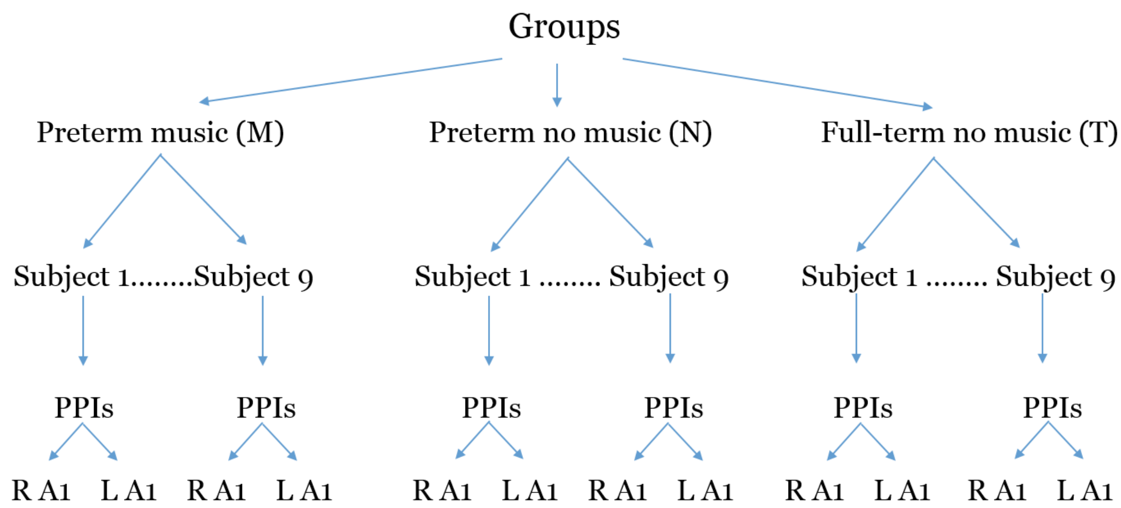

Figure 3: Flow chart representing the outline of the PPI analysis.

CHAPTER III: RESULTS

Psychophysiological interactions

Second level analysis: One-sample t-tests

Based on the second level analysis of the PPI results for the **Original music > Tempo modification** contrast and the left primary auditory cortex as seed region, we found statistically significant activation in the left middle cingulate cortex (MCC, $p<0.007$ FWE at cluster level) and in the right caudate nucleus and putamen ($p<0.01$ FWE at cluster level) for the Music group only. The same regions were identified when the right primary auditory cortex was used as seed region, but failed to pass the statistical significance test based on the corrected p-values ($p=0.275$ FWE). Interestingly, no statistically significant interactions were found for the Non-music and the Term group for both seeds (**Figure 4**).

In order to investigate further this discrimination capacity, we conducted a new standard PPI analysis using the **Original music > Key** modulation contrast. In this case, both the one-sample and two-sample t-tests showed no statistically significant activations for any group. Thus, there are no regions that increase their relationship/connectivity with the seed regions (left and right primary auditory cortex respectively) under the original music > key modulation condition.

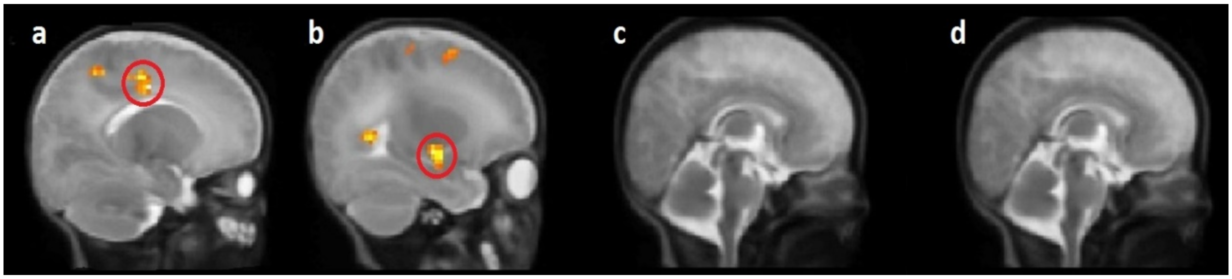

Figure 4: One-sample t-tests of PPI results for the Music group: Psychophysiological interaction (PPI) of the left primary auditory cortex (seed ROI) showing task-specific coupling. Areas that are coupled to the left primary auditory cortex for Original>Tempo are: (a) the left middle cingulate cortex (MCC, $p<0.007$ FWE at cluster level) and (b) the right caudate nucleus and putamen ($p<0.01$ FWE at cluster level). The statistically-significant clusters are pointed out by red circles. No significant activations were found for Non music (c) and Term group (d).

Second level analysis: Two-sample t-tests

Two-sample t-tests were performed on the PPI results in order to examine between-group differences across the experimental conditions. In more detail, two tests, one for each seed region, were performed for each pair of groups (Music vs Non-music, Music vs Term group and Non-music vs Term group).

The first two tests aimed to investigate group differences between the Music and Non-music group (Music > Non-music) for the **Original music > Tempo** modification contrast and the right and left primary auditory cortex as seeds, respectively. Based on the second level analysis of the PPI results (Music > Non-music, seed: right primary auditory cortex), we found statistically significant activation in the right thalamus ($p<0.009$ FWE at cluster level), left middle cingulate cortex as well as in the left caudate nucleus ($p<0.01$ FWE at cluster level for both clusters) (**Figure 5**). On the other hand, based on the two-sample t-test of the PPI results for the left primary auditory cortex as seed region, no statistically significant differences were found.

The next two-sample t-tests examined group differences between the Music and Term group (Music > Term) for the **Original music > Tempo** modification contrast and the left and right primary auditory cortex as seeds, respectively. Based on the second level analysis of the PPI results for the left primary auditory cortex as seed, we found statistically significant activations in the right and left superior temporal gyrus (STG, $p<0.05$ FDR and $p<0.03$ FWE at cluster level, respectively) and in the left middle cingulate cortex (MCC, $p<0.01$ FWE at cluster level). Additionally, statistically significant activations in the left middle cingulate cortex and putamen ($p<0.001$ FWE at cluster level for both clusters) were pointed out by the second level analysis of the PPI results for the right primary auditory cortex as seed (**Figure 6**).

The last series of two-sample t-tests which examined group differences between the Non-music and Term group (Non-music > Term) for the **Original music > Tempo** modification contrast and both left and right primary auditory cortex as seeds, showed no statistically significant differences between the two groups for the specific contrast and for both seed regions.

As mentioned before, both the one-sample and two-sample t-tests of the PPI results for **Original > Key modulation** condition, showed that there were no statistically significant activations for any of the three. Thus, there are no regions that modulate their relationship with the seed regions for the specific contrast.

Importantly, we also conducted a new PPI analysis for Tempo modification > Original music, but did not find significant interactions for any group. Additionally, in all cases we tested for brain activations using the opposite contrasts (Non-music > Music, Term > Music and Term > Non-music) but no statistically significant results were found.

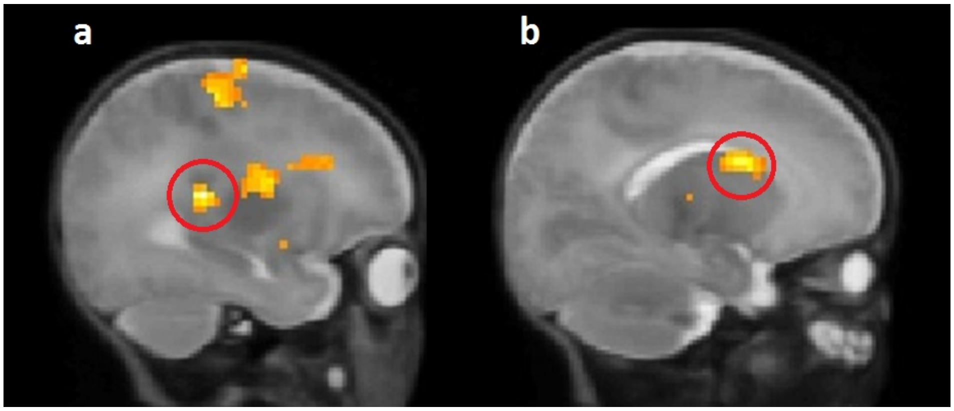

Figure 5: Two-sample t-tests of PPI results for the Music vs Non-music group: Psychophysiological interaction (PPI) of the right primary auditory cortex (seed ROI) showing task-specific coupling. Areas that are coupled to the right primary auditory cortex for Original>Tempo are: (a) the right thalamus ($p<0.009$ FWE at cluster level) and (b) the left caudate nucleus and middle cingulate cortex ($p<0.01$ FWE at cluster level). The statistically significant clusters are pointed out by red circles.

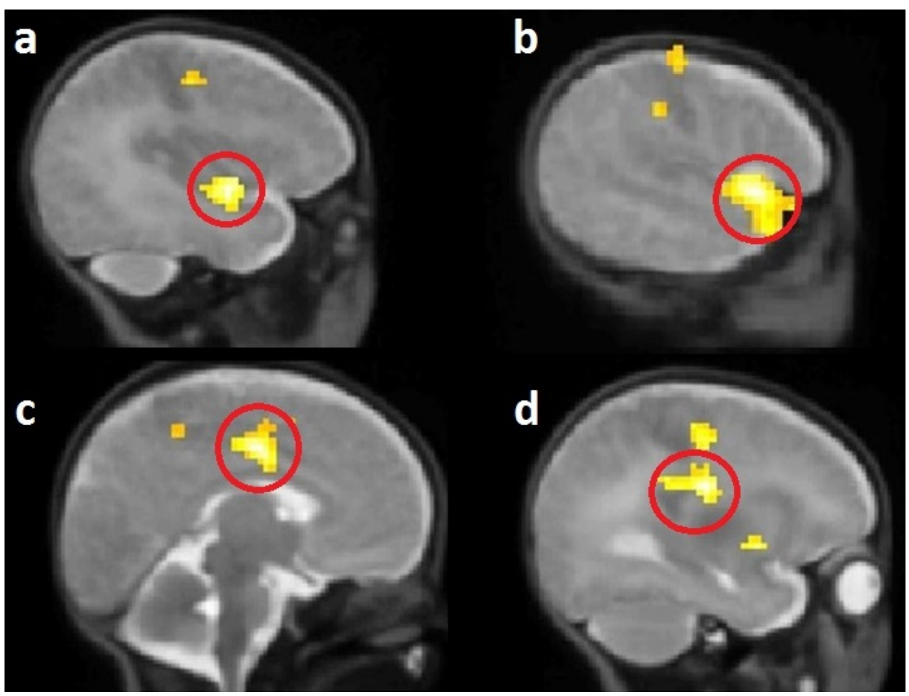

Figure 6: Two-sample t-tests of PPI results for the Music vs Term group: Psychophysiological interaction (PPI) of the left and right primary auditory cortex (seed ROI) showing task-specific coupling. Areas that are coupled to the left primary auditory cortex for Original>Tempo are: (a) the left superior temporal gyrus ($p<0.03$ FWE at cluster level), (b) the right superior temporal

gyrus ($p<0.05$ FDR at cluster level) and (c) the middle cingulate cortex ($p<0.01$ FWE at cluster level). Areas that are coupled to the right primary auditory cortex are: (d) the left middle cingulate cortex and left putamen ($p<0.001$ FWE at cluster level). The statistically significant clusters are pointed out by red circles.

CHAPTER IV: CONCLUSION AND DISCUSSION

Neural and functional consequences of prematurity warrant for consistent neonatal intensive care unit (NICU) enhancement. Appropriate environmental enrichment has been shown to have positive impact on behavioral states and brain structural connectivity (Als et al., 2004). Over the past decade, music in NICU has been shown to positively impact on physiological and behavioral measures (Halsbeck et al., 2012). In the context of the present study we investigated the impact of music intervention on preterm brain functional connectivity by performing psychophysiological interaction (PPI) analysis on fMRI data and showed that music in NICU can impact on the functional connectivity of the primary auditory cortex.

In the context of the present study, we investigated the impact of music intervention on preterm brain functional connectivity by performing psychophysiological interaction (PPI) analyses on fMRI data. As mentioned in the previous chapter, based on the one-sample t-test results we found significant activations (PPI effect) for the Preterm Music group (**Original music > Tempo modification**) but not for the Preterm Non-music and Term group (for both seed regions). Additionally, we conducted a new PPI analysis for **Tempo modification > Original music** but no statistically significant results were found for any group showing that this effect is not a simple beat/tempo detection ability. All these findings suggest task-specific increase in favor of the Preterm Music group in the exchange of information between the identified regions and the respective seed regions when listening to trained original music.

We could argue that these differences in music processing may rely on learning due to musical training during the hospitalization rather than beat detection ability**.** This argument is also supported by the two-sample t-tests on **Original music > Tempo modification** PPI results, since statistically significant results were only found for the Preterm Music vs Preterm Non-music and Preterm Music vs Term group comparisons. Adult musicians show a greater activity in MCC and STG for familiar music than adults without musical training (Groussard et al., 2010), indicating that activation of these regions for familiar music is dependent on musical expertise.

Thalamus and basal ganglia/striatum (including putamen and caudate nucleus) have been shown implicated in tempo processing in adults and lesion in this structure has been shown to impair tempo detection (Schwartze et al. 2011). Additionally, putamen has been shown to increase its functional connectivity with superior temporal gyrus (STG) when listening to beat stimuli compared to non-beat stimuli (Grahn 2009).

Increased functional connectivity between auditory cortex and striatum in Preterm Music group during Original music compared to Tempo modification music may reflect tempo processing of the known music (Schwartze et al. 2011). Unlike previous electroencephalography studies (Winckler et al., 2009; Hàden et al 2015), our results do not support the idea of an innate ability to detect temporal structure in sound as no difference of functional connectivity were observed in preterm and full-term newborn without music experience. Thus, this discrimination ability of the Preterm Music group may rely on learning due to music exposure during their NICU stay as mentioned before. Furthermore, all the three groups did not show higher levels of coupling between the primary auditory cortices and the rest of the brain under the Tempo modification condition compared to Original music condition. Thereby, Preterm Music group displayed a greater increase in functional connectivity between primary auditory cortices and left MCC, right caudate nucleus and putamen only when listening to the known music (Original music). Our results indicate that unlike Preterm-Non-Music and Full-term newborns, Preterm-Music infants process differently the original music, and thus, detect the differences of tempo between the Original music and the Tempo-modification music.

Furthermore, putamen and caudate nucleus activates more in beat prediction than beat detection (Grahn & Rowe 2012) and previous studies in adults displayed an increased activity in thalamus, putamen (Pereira 2011), superior temporal areas and MCC (Groussard et al., 2010) for familiar music. Thus, when listening to familiar music, Preterm Music infants may recognize the Original music as familiar and thus the higher increase in functional connectivity between primary auditory cortices and superior temporal gyrus, putamen and caudate nucleus in this group compared to Preterm Non-music group and Full-term group may reflect greater tempo processing for the familiar music (Original music) than for the Tempo modification condition. Furthermore, the increased coupling between primary auditory cortices and thalamus, MCC, putamen and superior temporal gyrus in Preterm Music group than in Preterm Non-Music or Full-term group supports the idea that Preterm Music infants recognize the music they heard during their stay in NICU (Original music) and remember its tempo.

Fetuses and full-term infants have been shown to remember sounds such as their mother's voice, music, phonemes and so on. Indeed, fetuses exhibited changes in their movements when played a tune heard previously during pregnancy (Hepper 2012). Furthermore, fetal auditory learning has been demonstrated in newborns when listening to the same music they heard during last trimester of gestation but with some notes changed. In this study, the exposed fetuses showed enhanced brain activity (mismatch negativity, ERPs) in response to pitch changes for the trained variants after birth (Partanen et al., 2013). Additionally, after one week of exposure 6 months old infants remembered the melody but also the tempo and timbre information of the music they heard (head-turn preference procedure) (Trainor et al., 2004). Another study showed that 2 to 4 month old infants could discriminate music that they are used to and music played 15% faster, based on observations of infants gaze direction (Baruch et al., 1997). However, no studies have tried to explore this auditory learning capacity in preterm infants using functional MRI and applying psychophysiological interaction (PPI) analysis to define the neuronal basis of such music training in the brain.

Finally, Preterm Non-Music group and Full-term group did not show statistically significant different levels of coupling between the primary auditory cortices and the rest of the brain in any condition supporting the idea that the differences in processing Original music and Tempo modification conditions, observed only in Preterm Music infants, are linked to their music exposure in NICU.

In order to investigate further this discrimination ability, we investigated the functional connectivity between left and right primary auditory cortex and the rest of the brain during Original music compared to Key Transposed music conditions. No difference was found in any group as well as between groups. It has been observed that pitch processing is already present in full-term newborn (Hàden 2009), and that newborn process differently music with key shift inside the melody (Perani 2010). However, in these studies key modulations were presented either as deviant or as alteration of the melody. On the contrary, in our study the entire stimulus is transposed on a different key and therefore the melody is identical. Thus, it is not surprising that Preterm Non-Music and Full-term infants process equally both Original music and Key Transposed music. On the other hand, the lack of differences in processing in Preterm Music group could be explained by the fact that they recognize the melody of the music independently of the key. Plantinga and Trainor (Plantinga & Trainor 2005) showed that key transposition does not affect melody recognition in 6 months old infants. Here, we show that even preterm infants at term equivalent age can remember a melody independently of its pitch. It seems that preterm infants do not retain precise absolute pitch information but can remember the melody and its tempo.

CONCLUSIONS

Taken together, these results indicate that enrichment of the NICU with music can have long lasting learning effects on music processing with an increased connectivity between primary auditory cortex and brain regions implicated in music tempo and familiarity processing. This study is the first one to show using fMRI that preterm newborn can **learn** melody discrimination by listening to music during their stay in neonatology. Our results suggest that NICU's environment can induce brain functional connectivity changes that are associated with learning. Thus, the NICU environmental stimuli need to be carefully and wisely chosen in order to be beneficial for the newborns.

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