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The relationship between acute stress and EEG repetition suppression in infants

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ABSTRACT

Over activation of the hypothalamo–pituitary–adrenal (HPA) axis in stress situations is known to influence learning and memory. In adults, an inverted-U shape relationship between acute stress, and learning and memory has been demonstrated. Whether this model fits learning performances in infants is unknown. In this study, we used EEG repetition suppression as physiological measure of learning and salivary cortisol in response to a stressor to investigate the relationship between acute stress and learning in infants. We hypothesized that EEG repetition suppression would be modulated by acute stress following an inverted-U shape relationship. Saliva samples were collected during an EEG experiment before, during and after EEG net installation in 37 healthy infants (18 males) aged between 6 and 26 months. The effect of variation in stress hormones on repetition suppression were modeled using a linear mixed model, with cortisol, age and sex as predictors. Results indicated that in healthy infants, elevations in stress hormones within the normal range are associated with a higher repetition suppression response and an increased response to the first presentation of the stimulus. The later increase could be related to vigilance. Considering that early childhood is a critical period of development, future studies should keep investigating the influence of stress on learning processes in infants.

1. Introduction

Stress, through hormones release, can affect learning and memory (Lupien and Lepage, 2001). More specifically, the relationship between acute stress and learning and memory has been shown to follow an inverted-U shape model, meaning cognitive functions are enhanced under stress levels that are not too low or too high. This inverted-U shape relationship has been well documented in adults (Lupien et al., 2005; Lupien and McEwen, 1997), however, it is unknown if learning and memory is similarly influenced by acute stress in infants. Considering that early life is a crucial learning period for later development, it is mandatory to have a better understanding of learning and memory modulators during this period.

When encountering a stressful situation, activation of the hypothalamic-pituitary-adrenal (HPA) axis leads to the release of glucocorticoids, predominantly cortisol in humans. Several structures with large numbers of glucocorticoids receptors, such as hippocampus, amygdala and prefrontal cortex, are important regulators of the HPA axis (Dedovic et al., 2009). The inverted-U shape relationship between acute stress and learning has been linked to the modulatory effect of stress hormones on long-term potentiation (LTP), a long-lasting increased synaptic efficacy mechanism (Teyler and DiScenna, 1987; Zoladz and Diamond, 2009). Acute stress leads to impaired long-term potentiation (LTP) and increased long-term depression (LTD) in the hippocampus, which results in a prolonged reduction in synaptic efficiency involved in hippocampus-dependant functions (Kim and Diamond, 2002; Wong et al., 2007). Adult data indicates that when all receptors are saturated under high stress levels, learning is impaired.

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On the contrary, learning is enhanced under low stress levels since both types of receptors are not fully saturated (Lupien et al., 2005; Zoladz and Diamond, 2009).

Studies on the effect of acute stress on learning in infants have been hindered by the difficulty to measure learning in this population. Infant EEG experiments offer the possibility to examine neural learning responses more precisely in developmental studies. Repetition suppression, in particular, is a well-documented phenomenon thought to reflect learning mechanisms (Bouchon et al., 2015; Nordt et al., 2016; Snyder and Keil, 2008). Repetition suppression refers to the reduction of neural activity in response to the repeated presentation of a stimulus (Schacter and Buckner, 1998). This phenomenon, observables in all age groups, is present at many cortical levels and can be assessed using multiple functional neuroimaging techniques, such as EEG (Grill-Spector et al., 2006). In developmental research, this phenomenon is referred to as neural habituation (Nordt et al., 2016; Sirois and Mareschal, 2002). An early ERP repetition study by Dehaene-Lambertz and Dehaene (1994) demonstrated strong repetition suppression effects in very young infants. They presented two-to-three-months-old children with sequences of five syllables and found a positive peak around 400 ms that decreased in amplitude at the second presentation of the stimulus at posterior temporal channels. Moreover, it has been proposed that neural habituation is the correlate of behavioral habituation reflecting a form of learning (Turk-Browne et al., 2008). This form of learning plays a major role in perceptual and cognitive development in early childhood (Sandman et al., 1997). Thereby, a study by Snyder and Keil (2008) showed that a reduction of activity in the posterior regions during an encoding phase of faces and objects was associated with a looking preference for novel compared to familiar stimuli.

Vigilance, an arousal state operating through the HPA axis (Hancock, 1989), could play a role in the habituation response. Vigilance refers to the ability of orienting and maintaining attention to a task and remaining alert to stimuli for a certain period of time (Parasuraman, 1986). The relationship between cognitive performances and states of cortical activation has been described as an inverted-U shape, such that performance is enhanced during arousal states that are neither too high nor too low (Oken et al., 2006). With decreased vigilance, decreased event-related potentials amplitudes and a decreased orienting response were found (Oken et al., 2006).

Early childhood is a critical period for brain development, especially for the development of brain structures involved in learning and memory. The stress-learning relationship and the brain mechanisms affected by acute stress during the first year of life are still unclear. Here, we investigated how variation in stress hormones, measured through salivary cortisol collected after a potentially stressful situation, affects EEG repetition suppression in infants. We hypothesized that the relationship follows the same inverted-U shape model as found in adults. We expect to find greater repetition suppression response under normal cortisol levels and poorer repetition suppression response under very low and very high cortisol levels.

2. Method

2.1. Participants

Forty-eight (25 males) healthy infants aged between 6 to 26 months (mean age ± SD: 15.77 ± 6.03) participated in the study. Families were recruited in day cares and through social networks. Developmental information was gathered from an in-house developmental questionnaire completed by the parents. All infants were born at term with no significant pregnancy or delivery complications, had no significant health problems or suspicions of developmental delay. Parents gave informed written consent prior to the study. The study was approved by the ethics, administrative and scientific committees of the Ste-Justine's University Hospital Research Center. EEG data of five participants was rejected because of excessive movement artefacts, four participants were excluded from the analyses because they had incomplete salivary cortisol data and two others were excluded because of potentially contaminated saliva samples, and because of extreme scores (+3 Z score) on multiple variables regarding saliva protocol characteristics, respectively. Therefore, the final sample was composed of 37 infants (18 males; mean age ± SD: 14.78 ± 5.63).

3. Materials and procedure

3.1. Cortisol

Cortisol reactivity to stress was assessed using salivary cortisol. Saliva samples were collected using Salivette device (SalivaBio Children's Swab; Salimetrics LLC, Carlsbad, CA). A sterile synthetic swab was placed in the participant's mouth for intervals of 15 to 30 s, for a total of 60 to 90 s. It was then placed in a pierced tube, fitted in an external tube. Installation of the EEG net served as the stressor since exposure to medical procedures have been shown to lead to increased stress reactivity in children, as assessed through stress hormone levels (Gunnar and White, 2001; Lupien et al., 2011). Furthermore, EEG evaluation was a novel experience for the participants. To prevent children from pulling on the EEG net, parents had to restrain their arms which could lead to frustration and implied reduced parental support during the stressful situation (Gunnar et al., 2009). Saliva samples were collected before entering the EEG exam room, 20 min and 45 min following the stressor. In order to prevent any contaminations or spike in cortisol levels, parents were asked to not feed their infants throughout the experiment. The mouth was rinsed with water prior to sample collection when participants had taken food or put an object (i.e. toys, pacifier) in their mouth. Only five participants ate during the testing and two participants took medication (i.e., acetaminophen or antibiotics) the day of the testing. Stress measures of these participants were not statically different from the other participants ($Z < 3$). Inter-assay CV for our analyses varied from 2.75 to 6.53%, while only two samples had a CV over 15%. Single cortisol values were used in 12.4% of samples and no sample included in the analyses were rerun at dilution since the samples that were rerun still showed significant outlying values.

To reduce bacterial growth, saliva samples were frozen at −80 °C until further analysis. Samples were then brought to room temperature and centrifuged at 15,000 $g \times 15$ min. Saliva was analysed with a high sensitivity enzyme immunoassay kit (Salimetric State College, PA, catalogue number 1-3102.) according to manufacturer's instructions. Detection ranged from 0.0012 µg/dl to 3 µg/dl. Samples showing an out of curve reading were rerun at a 1:4 dilution. Samples were also rerun if the intra assay coefficients of variation (CV) were > 15%, unless the difference between the two values was below 0.03 µg/dl. Samples with enough volume were run in duplicate. All samples for a participant were run in the same batch, and each batch included samples from male and female infants of different ages.

3.2. EEG learning task

The task consisted of audio-visual stimuli featuring a woman and a man alternating in the articulation of the vowels /a/ or /i/ (Basirat et al., 2014). The audio-visual design served to maximize infants' attention. Stimuli were generated by a Dell optiplex 790 PC using E-Prime 2.0 software (Psychology Software Tools Inc., Pittsburgh, PA, USA) on a 17" screen placed at a viewing distance of 60 cm. The sounds were delivered through two speakers located laterally at 30 cm from the infants' ears. Infants were seated on their parents' lap. The onset of the auditory vowel coincided with a visual clip lasting 200 ms of the mouth fully opened (frame 1). Following the end of the sound, two frames of 60 ms showing the mouth gradually closing were presented. During the next 280 ms, the face with a closed mouth was presented followed by the onset of the next vowel (Fig. 1). The initial learning phase

Fig. 1. Experimental design. Task consisted of man or a woman (pictured) articulating the vowel /a/. On each trial, infants were presented with three consecutive /a/ while de fourth vowel could be either /a/ or /i/. The sound lasted 200 ms, in synchrony with the first frame (mouth opened). Following the end of the sound, two frames of a mouth gradually closing was presented (60 ms). Finally, during the last 280 ms, a face with a closed mouth was presented.

comprised 16 trials respecting a xxxY (aaaI) specific rule, called "standard". During the test phase, out of 80 trials, 75% followed the xxxY (aaaI) specific rule. The other 25% followed a xxxx (aaaa) rule, called "deviant". To facilitate learning, all deviant trials were followed by a standard trial. To assess repetition suppression response, the first three /a/ of each "standard" trial, apart from those following a "deviant" trial, were analysed.

3.3. EEG recordings

EEG recordings took place in a dark soundproof experimental chamber. EEG was recorded continuously with an high-density EEG system containing 128 electrodes (Electrical Geodesics System Inc., Eugene, OR, USA). Signals were acquired and processed by a G4 Macintosh computer using NetStation EEG Software (Version 4.5.4). Data were acquired at a 1000 Hz sampling rate and analog bandpass filter of 0.1–4000 Hz was applied. Impedances were kept bellow 40 kΩ (Tucker, 1993). Off-line signal processing and analyses were performed using MATLAB (Mathworks, Inc., Natick, MA) and the EEGLAB toolbox (Delorme and Makeig, 2004). Data were digitally filtered with a lower-bound 0.5 Hz and a 60 Hz notch filter. Twenty-eight electrodes containing muscular artefacts placed around the neck and face were excluded. Channels presenting voltage lower than 2 μV and higher than 200 μV were rejected. Data was re-referenced to an average reference. Eye movement artefacts were rejected using semi-automatic independent component analysis (ICA) as implemented in EEGLAB toolbox (Delorme and Makeig, 2004). Visual inspection of the segmented data (-1000 to 2500 ms related to onset of the first vowel in a trial) was performed to manually reject epochs with significant artefacts. Following epochs exclusion, an average of 57/80 (SD = 10.9) epochs were kept for analyses.

3.4. Event-related potentials

Auditory evoked potentials analyses were conducted as they are widely used in developmental studies. In infants, the most prominent auditory response is a large positive component (P2) with a maximum amplitude recorded at frontocentral sites at around 200–250 ms (Lippé et al., 2009; Picton and Taylor, 2007), followed by a large negative component (N2) (Lippé et al., 2009). Thereby, ERP analyses were conducted on the auditory P2 and N2 components. We first segmented and cleaned the data in 3500 ms time windows to ensure that each /a/ had the same numbers of segments. Each epoch was then segmented into three segments representing the time-window of each stimulus (a_1: 0–600 ms, a_2: 600–1200 ms and a_3: 1200–1800 ms) and a baseline correction (-200-0 ms) was applied on the 800 ms time window. Peaks were obtained by averaging separately the first, second and third /a/ (a_1-a_2-a_3). A region of interest (ROI) was created by selecting five electrodes in the FCz region (E6, E7, E13, E106, E112) since this region showed the highest P2 amplitude. Amplitudes were defined using a peak-to-peak measure between P2 and N2 and amplitudes were measured at the mean value extracted from the ROI. Since one participant had an extreme P2/N2 peak-to-peak amplitude value on the first /a/, instead of excluding it from the analyses, we chose to winsorized the data, so the value was lowered to the highest normal value of the set.

4. Statistics

4.1. Preliminary analyses

Preliminary analyses were conducted to assure there were no differences between male and female regarding important variables such as age and saliva protocol characteristics. T-tests were used to verify if the stressor produced a significant increase in cortisol concentrations. Finally, Pearson's r correlations were performed to see if age was correlated with the mean amplitude of each /a/ on the two components and with cortisol measure.

4.2. Cortisol analyses

The averaged cortisol concentration was used for samples run in duplicates and single concentration was used for samples with insufficient volume for duplication. Due to the small sample size, values of saliva samples with abnormally high concentration were also winsorized. To assess stress reactivity, area under the curve with respect to increase (Pruessner et al., 2003) was used as stress measures. The area under the curve with respect to increase (AUC_I) is calculated by using a derived of the trapezoid formula in which cortisol levels for each sample and time between each measurement is considered (Reinhardt and Soeder, 2001). Advantages of using an AUC_I formula include using cortisol levels of the first sample as the baseline, thus assessing the change in reactivity over time rather than the total hormonal response and controlling for individual differences in baseline cortisol levels and cortisol's circadian rhythm. In order to test our hypothesis of an inverted-U shape relationship between cortisol levels and EEG repetition suppression, the AUC_I measure was squared. For all stress measures, positive values indicate an increase while negative values indicate a decrease in cortisol concentrations. Higher absolute values suggest greater changes in cortisol concentrations throughout the experiment.

4.3. Linear mixed model

Statistical analyses were performed using SPSS statistics, version 25 (IBM Corp., Armonk, NY, USA). Linear mixed models (LMM) were used to assess how cortisol level predicted changes in cerebral activity across repetitions. LMM analyses were chosen because they can deal easily

with small sample size, in addition to enabling random intercepts and slopes, allowing for nonlinear modeling (quadratic and cubic) and selecting appropriate covariance structures (Field, 2013; West, 2009).

Linear trajectories were modeled individually to estimate the participant's initial cerebral activity (intercept), the slope of participant's cerebral activity trajectory and error. Each measure of cortisol level was tested in a different model. Maximum likelihood (ML) was used to estimates all the models and the identity covariance structure was used, which is a scaled identity matrix, since it had the best model fit compared to other available structures. Also, the best model fit was found while allowing the intercept but not the slope to vary randomly in our models. Finally, age and sex were added sequentially to the models and a chi-square likelihood ratio was used to verify model fit improvement.

5. Results

5.1. Preliminary analyses

Preliminary analyses showed no significant differences between male and female infants regarding age, time of the day when testing was conducted, time before the stressor, total time of testing and cortisol variables. Results of the T-tests showed a general decrease in cortisol concentrations over time, though the difference were not significant, suggesting that infants' cortisol concentrations were stable throughout the experiment (Fig. 2). Age was not correlated either with the mean amplitude of each /a/ on the two components and cortisol measure. Table 1 display the descriptive statistics of cortisol predictors.

5.2. Linear mixed models

5.2.1. Baseline models

We first tested the unconditional mean model to serve as baseline in order to examine individual differences in amplitude across stimulus repetition without including time as a variable. This model gives the intraclass correlation coefficient (ICC), which indicate the amount of variance in the outcome that is related to interindividual differences. The interclass correlation coefficient was 0.25, indicating that 25% of the total variation in amplitude was due to interindividual differences, thus justifying the use of linear growth model (Heinrich and Lynn, 2001).

Secondly, we tested the unconditional linear growth curve model. This model focused on individual growth curves, more specifically individual change over time and served as a baseline for comparing the subsequent models. By using the chi-square likelihood ratio test to compare the mode fit, the best model was found by allowing the intercept but not the slope to vary randomly so only this parameter was retained in the following models. The average (SE) initial P2/N2 peak-to-peak amplitude was 6.47 μV (0.75) (intercept) and the average (SE) slope was -0.86 (0.32).

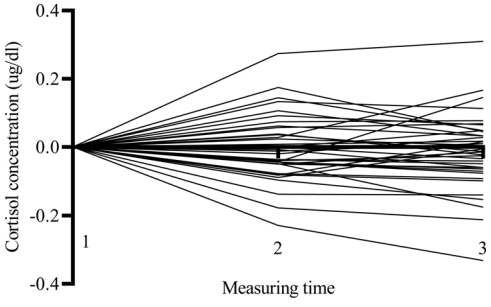

Fig. 2. Spaghetti plot of cortisol concentrations across the experiment. This graphic shows the plotted curve of each participant's cortisol concentrations across the three measuring times. The model was baseline-adjusted (Sample 1 = 0) and show differences in cortisol concentrations between the three measuring times (Sample 2 = sample 2-sample 1, Sample 3 = sample 3-sample 2).

Table 1
Descriptive statistics of cortisol predictors.

	Mean	Standard deviation
AUC_I	−0,32	3,66
AUC_I^2	13,15	25,09

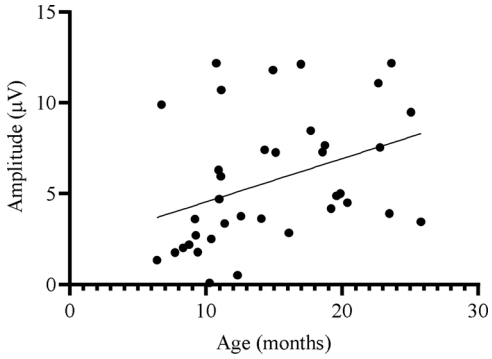

Fig. 3. A) Effect of age on the auditory P2/N2 peak-to-peak measures on the first /a/ of each trial. This figure shows that P2/N2 peak-to-peak measures on the first /a/ of each trial increases with age.

5.2.2. Predictors

5.2.2.1. Age. When adding age to the unconditional model, model fit was significantly improved (χ^2 (2) = 7.3, $p < 0.05$). Age was not a significant predictor of the linear changes in amplitude across repetitions (F (1, 105.2) = 0.994, p = 0.321). However, age was significantly associated with the intercept (F (1, 106.7) = 5.5 p = 0.021), suggesting that the initial P2/N2 peak-to-peak amplitude was higher for older infants (Fig. 3). Therefore, age was retained in the subsequent models.

5.2.2.2. AUC_I. AUC_I was added as a predictor in the unconditional model as well as the interaction between repetitions and AUC_I. Adding AUC_I as a predictor improved the model significantly (χ2 (2) = 15.5, $p < 0.001$). Results indicated that change in cortisol response was positively associated with the intercept (F (1, 104.8) = 15.15, $p < 0.000$) (Fig. 5). Results also indicated the predictor had a significant negative effect on the linear changes in amplitude across repetitions (F (1, 74) = 8, p = 0.006) (Fig. 4). Age was still significantly associated with the intercept (F (1, 104.8) = 7.8, p = 0.006)

5.2.2.3. AUC_I^2. AUC_I^2 was added as a predictor in the unconditional

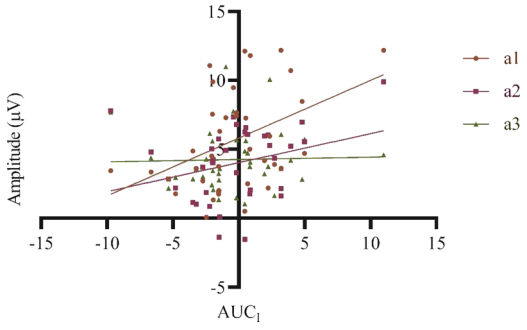

Fig. 4. Effect of cortisol AUC_I on the three presentation of the stimulus. This figure shows that infants with greater increases in cortisol concentration had greater repetition suppression response. Thereby, infants with greater increases in cortisol concentration showed greater amplitude decreases across the three presentation of the stimulus.

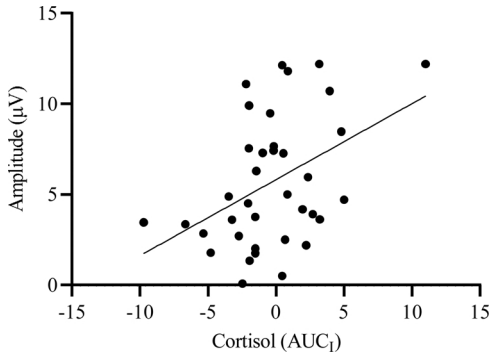

Fig. 5. Effect of cortisol concentrations on the auditory P2/N2 peak-to-peak measures on the first /a/ of each trial. This figure shows that infants with greater increases in cortisol concentrations during the experiment, as measured with the AUC_I, had amplest P2/N2 peak-to-peak measures on the first /a/ of each trial, while infants with smaller increases in cortisol concentrations during the experiment showed less ample P2/N2 peak-to-peak measures on the first /a/ of each trial.

model as well as the interaction between repetitions and AUC_I^2. Adding AUC_I^2 as a predictor did not improved the model significantly ($\chi 2$ (2) = 3.4, $p > 0.05$). Moreover, AUC_I^2 was not associated with the intercept (F (1, 105.2) = 0.994, p = 0.321) and was not found to be a significant predictor of the linear changes in amplitude across repetitions (F (1, 74) = 0.015, p = 0.904).

5.2.2.4. Sex. Sex was added to the models as a predictor as well as the interaction between sex, stress measures and repetitions. This predictor did not improve model fit for any of the models. Moreover, sex was not associated with the intercept and was not found to be a significant predictor of the linear changes in amplitude across repetitions in any model.

6. Discussion

The aim of this study was to investigate how acute stress in infants affects the EEG learning response. Learning was measured using a repetition suppression paradigm and acute stress was assessed by salivary cortisol. Our results first suggested that our experiment did not induce elevated levels of stress in our participants, suggesting that EEG was not a significant stressor for the infants. Nevertheless, our results indicated that elevations of stress hormones within the normal range was associated with higher auditory P2/N2 peak-to-peak measures on the first /a/ of each trial. Hence, we posit that increases in cortisol concentration during the experiment resulted in an augmented orientation response. Our results also suggested that elevations of stress hormones within the normal range was associated with a stronger repetition suppression response and that this relation did not follow an inverted-U shape. Further, the results indicated that infants showed a repetition suppression response that was not affected by age nor by sex.

6.1. Repetition suppression

Our results suggested that in healthy infants, repetition of the same auditory stimulus generated an EEG repetition suppression response, which attenuated after the second presentation of the stimulus. This finding is in accordance with the existing literature positing that the second presentation of the same syllable results in a decreased component amplitude, whereas additional presentations of the syllable do not lead to further decrease (Dehaene-Lambertz and Dehaene, 1994). Several factors are believed to influence the repetition response, such as attention, expectation, stimulus recognition, explicit memory and learning (Segaert et al., 2013) and the number of repetitions (Müller et al., 2012). The direction of the repetition response can also vary between infants depending on their processing capacities (Nordt et al., 2016). Our study demonstrated that elevations of stress hormones within the normal range are another factor influencing EEG repetition effect in infants, more specifically on the first presentation of the stimulus.

6.2. Orienting response

Our results indicated that greater increases in cortisol concentration were generally associated with amplest auditory P2/N2 peak-to-peak amplitudes in response to the first /a/ of each trial. In our study, infants with greater increases in cortisol concentration during the experiment showed an elevated response to the first /a/ of each trial compared to infants with smaller increases in cortisol concentration. This result could be explained by the role of the HPA axis in vigilance. Participants with greater increases in cortisol concentration might have been more vigilant towards their environment and more alert to the new stimulus whereas participants with smaller increases in cortisol concentration might have been less vigilant and displayed the usual decreased amplitude associated with decreased vigilance (Oken et al., 2006). This would mean that the first stimulus of each trial elicited a stronger orientating response in infants with greater increases in cortisol concentration, suggesting that increases in cortisol concentration following exposure to a novel event were associated with the orienting response.

Moreover, our results indicated that increases in cortisol concentration influenced the repetition suppression response. Indeed, infants with greater increases in cortisol concentration throughout the experiment showed greater amplitude decreases between each presentation of the stimulus. Thus, learning was enhanced under normal elevation of stress hormones in infants, probably, again, du to the role of the HPA axis in vigilance. Infants who showed greater increases in cortisol concentration also showed amplest auditory P2/N2 peak-to-peak amplitudes in response to the first /a/ of each trial and then habituated faster to the stimulus, resulting in greater repetition suppression responses. It is important to note that in our study, the relationship between cortisol concentration and learning did not follow an inverted-U shape and was instead linear. It has to be taken into account that infants in our study showed no statistically significant cortisol increase, possibly explaining why the inverted-U shape relationship was not found in our very young participants. The adult model results from a significant increase of stress hormones in the system, modulating LTP and LTD in the hippocampus (Pavlides et al., 1995). Thus, the stress response induced by the EEG net installation might have been too small to modulate these mechanisms.

Despite our effort to limit parental comfort during EEG net installation, parents were still able to comfort their infant during the experimentation (e.g. by talking to them), explaining why infants were not significantly stressed. Indeed, infant's access to supportive adult care helps to buffer the activity of the HPA axis (Gunnar and Quevedo, 2007). A review of Gunnar et al. (2009) looked into the effectiveness of stressor tasks in elevating cortisol levels in infants. For infants between four to nine months of age, stressor paradigms are barely successful in elevating cortisol levels and it is even worse for infants from twelve to twenty-four months of age. During the first year of life, many infants display no cortisol increase to stressors that typically cause important behavioral distress reactions (Gunnar and Quevedo, 2007). Neurodevelopmental changes affecting reactivity and regulation of the HPA system during infancy, such as improved negative feedback regulation of the axis and decreased sensitivity of the adrenal cortex to ACTH, could partially explain the diminution of the HPA responsiveness and the variability in effectiveness of stressor tasks (Lashansky et al., 1991).

6.3. Age effect

In our study, age was also associated with an ampler auditory P2/N2 peak-to-peak amplitude in response to the first /a/ of each trial.

Older infants showed a greater P2/N2 peak-to-peak measure compares to younger infants. These results could be explained by the effect of maturation on the morphology, amplitude and latencies of auditory components (Fox et al., 2010). Indeed, electrophysiological activity is known to change with age (Kushnerenko et al., 2002). As the ERPs peaks sharpen, the peak-to-peak amplitudes increase, explaining the age effect found in our study on the auditory P2/N2 peak-to-peak amplitude in response to the first /a/ of each trial. Moreover, as infants get older, they become more vigilant and alert to their surroundings, meaning that age may also relate with the orienting response (Ruff et al., 1990).

6.4. No sex effects

This study did not find sex to be a significant predictor of the linear changes in amplitude across repetitions, nor was associated with the initial auditory P2/N2 peak-to-peak amplitude. Few controlled stress studies have been conducted in children, and the little available data is contradictory. Some studies find no sex differences in stress response while others seem to find the same pattern of sex differences in infants as in adults (Kudielka and Kirschbaum, 2005). In adults, stress in men is associated with activity in frontal regions and is highly correlated with cortisol levels whereas stress in women principally activates the limbic system and it is less correlated with cortisol levels (Wang et al., 2007).

6.5. Study design and limitations

The use of the repetition suppression paradigms as learning measure provides multiple advantages. First, repetition suppression paradigms follow principles similar to habituation paradigms. In both cases, the aim is to measure the decrease in neural activity following the repeated presentation of the stimulus. Both paradigms suggest that infants build a memory trace for the repeated stimulus and that they can discriminate dimensions and features of the stimulus (Turk-Browne et al., 2008). Moreover, repetition suppression paradigms can be conducted without collecting overt behavioral responses enabling data collection in populations, such as infants, in which active response tasks cannot be realized (Nordt et al., 2016). In developmental studies, salivary cortisol is widely used as a stress measure since it is a non-invasive method for assessing cortisol levels (Gunnar and White, 2001). Precautions were systematically taken (i.e. sustained parental arm restraint, reduced parental support) to increase the stressor effectiveness and to provoke individual differences in stress response. However, our stressor did not succeed in inducing elevated stress in all participants. Future studies should try different types of stressors to verify whether it is possible to generate a stress response large enough to modulate hippocampus-dependant mechanisms and assess if the increase in cortisol levels has the same inverted-U shape effect on learning as found in adults. Moreover, other stress indicators, such as skin conductance or behavioral measures should be use in addition to cortisol measurements, to assess stress response in infants.

7. Conclusion

This study has shown that elevations of stress hormones within in the normal range affect vigilance and the EEG learning response in infants. Greater increases in cortisol concentration during the experiment were linked to an increased response on the initial stimulus of each trial and were associated with greater EEG repetition suppression responses. Since little is known about acute stress and learning in infants, future studies should keep investigating this relationship in order to better understand the risk factors that can affect this crucial learning period.

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Declaration of interest

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