Protective teaching mechanisms in case of mild perinatal adversity

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Chapter 6

Stress reactivity during challenging educational situations in children born late preterm

Introduction: In kindergarten, late preterm children show increased susceptibility to structured and guided learning environments. One explanation for this observation might be found in increased levels of stress reactivity in late preterm children. A direct link between late preterm birth and increased levels of stress reactivity has however not been established. *Methods:* Kindergarten children (*N* = 250) took part in an educational test session administered by the researchers. During this session for each child a set of three saliva samples was collected, from which levels of the stress hormone cortisol were determined. Patterns of stress reactivity were compared between children born late preterm and their full term peers. *Results:* Children born late preterm show increased levels of stress reactivity. In anticipation of the unfamiliar test situation, cortisol levels are high. However, cortisol levels drop quickly when the test situation turns out to be highly structured and guided. In children born full term no fluctuations in cortisol levels between measurements were found. *Conclusion:* Children born late preterm show increased levels of stress reactivity. In unfamiliar situations they seems to show an increased stress response, while in guided and structured situations reduced levels of stress are experienced. Offering late preterm children an educational environment with high(er) levels of guidance and structure could thus facilitate learning in this group.

Children born late preterm (between 34^{th} and 38^{th} week of pregnancy) are at increased risk for experiencing a range of difficulties later in life. These children are more likely to encounter health problems, socio-emotional problems, and cognitive problems (Morse, Zheng, Tang, & Roth, 2009) which can result in diverse academic delays. At ages five and seven late preterm children experience more problems in both numeracy skills and word reading than their full term peers (Poulsen et al., 2013). Although predictive for academic delays, recent research suggests however that late preterm birth cannot be straightforwardly considered a vulnerability factor for such problems, but might be considered a plasticity factor (Van der Kooy-Hofland, Van der Kooy, Bus, Van IJzendoorn, & Bonsel, 2012; Merkelbach et al., 2018). These recent studies show that children born late preterm show increased susceptibility to the qualities of their educational environment, both for better and for worse.

Digital interventions offering five year old children structure, repetition of assignments, continuous and adaptive feedback, and guidance, resulted in late preterm children outperforming their peers (Merkelbach et al., 2018). These results were achieved with low intensity interventions: children only worked with the digital programs once a week for ten minutes, over a period of just two to three months. Results were comparable for both an intervention promoting early literacy skills (i.e. *Living Letters,* Van der Kooy-Hofland et al., 2012; Merkelbach, et al., 2018) and an intervention promoting early numeracy skills (i.e. *Clever Together,* (Merkelbach, Plak, & Rippe, under review). When assigned to a digital control condition which did not contain the same essential features, late preterm children fell behind as compared to their peers. This pattern of experiencing exceptionally positive outcomes in positive environments and exceptionally poor outcomes in negative environments is described by the *differential susceptibility model* (Belsky & Pluess, 2009): children born late preterm can thus be considered highly susceptible to the qualities of the interventions. Full term children however were not affected: they did not benefit nor experience disadvantage from working with *Living Letters* or *Clever Together*. Which biological mechanism explains why late preterm children are especially susceptible to some or all of the qualities central to *Living Letters* and *Clever Together*, remains however unknown.

A potential explanation for why late preterm children responded especially well to interventions containing structure, repetition of assignments, continuous and adaptive feedback, and guidance, might be found in dysregulation of the stress response in these children. A recent study reported on increased dispersion of the level of the stress hormone cortisol in hair samples, a reliable indicator of cortisol release over an extended period of time (D'Anna-Hernandez, Ross , Natvig, & Laudenslager, 2011), in late preterm children and children born small for gestation age (i.e. children with mild perinatal adversities) as compared to children who did not experience such adversity (Windhorst,

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et al., 2017). Children who had experienced mild perinatal adversities showed decreased levels of hair cortisol in case of high levels of maternal harsh parenting. However, when maternal harsh parenting was low, high levels of hair cortisol were found in this group. For children without mild perinatal adversities parenting style was not predictive of hair cortisol levels (Windhorst, et al., 2017). Since chronic stress can result in down regulation of the *Hypothalamus-Pituitary-Adrenal axis* (HPA-axis) (Fries, Hesse, Hellhammer, & Hellhammer, 2005), the bodily mechanism controlling the secretion of cortisol (Kolb & Wishaw, 2009), low levels of hair cortisol could indicate high levels of chronic stress. It could thus be assumed that the susceptible group experienced higher levels of stress in negative environments (i.e. high levels of harsh parenting) and lower levels of stress in positive environments (i.e. low levels of harsh parenting) than their non-susceptible peers.

Late preterm children may experience high levels of stress in both negative home environments and negative educational environments. On the other hand, a positive educational environment, as offered by programs like *Living Letters* and *Clever Together*, could result in a decrease of experienced stress in this group. High levels of stress could cause children to shut themselves from learning experiences, suggesting that lowering these stress levels would facilitate learning. However, for these programs to influence acute stress levels, directly facilitating learning, children late preterm would have to be able to show rapid and substantial changes in experienced stress, and thus experience increased levels of *stress reactivity.*

From an evolutionary perspective, increased levels of stress reactivity in late preterm children would be plausible, too. Talge, Neal, & Glover (2007) state that having high levels of stress reactivity can be evolutionary adaptive, because this trait can be useful when living in harsh and unpredictable environments. High stress reactivity facilitates rapid bio behavioral changes (e.g. increased heart rate) that prepare the body for direct action (Sapolsky, 2015). Already while still in the uterus, the fetus can receive cues about the quality of the environment (s)he will grow up in. High levels of maternal stress for example have a signaling function to the fetus, predicting an unsafe environment (e.g. Pike, 2004) in which high stress reactivity could thus be desirable. Through the process of *early programming* changes in the uterus due to maternal stress can evoke changes in the HPA-axis of the fetus (Matthews, 2002), resulting in these higher levels of stress reactivity. At the same time, high levels of maternal stress can also increase chances of late preterm birth (Dole, et al., 2002). Because high levels of maternal stress predict for both increased stress reactivity of the child and late preterm birth, considerable overlap between these factors would be likely. To our knowledge however, this association has not been evaluated.

In this study we compared acute stress responses between late preterm children and their full term peers in an educational environment. We hypothesize that children born late preterm will show elevated cortisol levels in anticipation of a potentially stressful event (i.e. being taken out of the classroom by an unfamiliar test assistant in order to complete performance tests) and a pronounced drop in cortisol levels when the child is consequently placed in an environment comparable to *Living Letters* and *CleverTogether,* in this case a testing environment offering structure, repetition of assignments, continuous and adaptive feedback, and guidance. We expected no changes in stress levels of full term children. Since acute changes in stress cannot be measured with the use of hair cortisol, we used successive salivary cortisol levels as an indicator of stress reactivity (e.g. Blair, Granger, & Peters Razza, 2005). To this end, salivary cortisol levels were determined at the start, halfway, and at the end of the test session.

Method

Design

The current study is embedded in a large scale study on the effects of digital educational programs in five-year-olds: *What Works for Whom* (e.g. Plak, Merkelbach, Kegel, Van IJzendoorn, & Bus, 2016). The project consisted out of two successive research waves (2012/2013 and 2013/2014) in which a total of 1972 children participated. The current sample (N = 440) was randomly selected from the group of children participating in the second research wave (N = 1083). As a critical test of stress reactivity, saliva samples were collected during a one-on-one test situation lasting on average almost 35 minutes (M = 34.81, SD = 7.14).

Participants

The subsample of 440 kindergarten children was subjected to an additional test session that took place after the intervention period, during which a series of three saliva samples was collected. Children from 63 different schools across the Netherlands were randomly selected for additional testing. Complete data for testing stress reactivity patterns were available for 250 children (see flow diagram of data attrition in Figure 1).

Procedure

After the intervention period (in which efficacy of e.g. *Living Letters* and *CleverTogether* was explored) conducted in the context of the overarching research study, trained test assistants, instructed to be guiding, supportive, and to offer continuous feedback, visited a selection of participating preschool classrooms for additional individual testing. Testing took place in a quiet, separate room in the school. During test sessions, children completed several tasks related to literacy skills and impulse control. Saliva samples were collected before, halfway through, and at the end of this test session that lasted about 35 minutes.

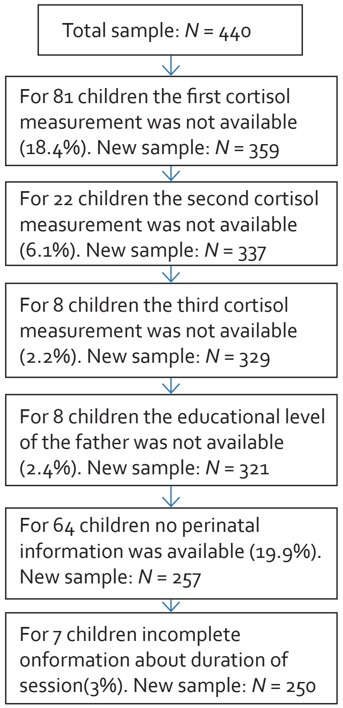


Figure 1. Flow diagram data attrition

Measurements

Perinatal information – The Netherlands Perinatal Registry (Stichting Perinatale Registratie Nederland, 2011) contains comprehensive data on pregnancy, pregnancy care (interventions, referrals), and pregnancy outcomes. These variables are recorded by the health care provider during prenatal care, delivery, and the neonatal and lying-in period. The register covers approximately 96% of all deliveries in the Netherlands. The data from three registers (the National Obstetric Database by midwives, the National Obstetric Database by gynecologists, and the National Neonatal/Pediatric Database) are annually sent to the national registry office, where a number of range and consistency checks are conducted. The perinatal registry can be accessed by researchers, provided that they have the written permission of the mother. Missing values in our sample were largely due to non-consent for retrieving data (61%). Attrition was also due to failure of connecting

registry data to the research database (39%). Criterium for being classified as born late preterm was to have a gestational age at birth of 34 - 37 weeks and 6 days.

Stress reactivity

*S*tress reactivity was derived from the cortisol levels in saliva samples. Since cortisol levels in saliva tend to lag behind five to twenty minutes, and to peak ten to thirty minutes after exposure to a stressor (Kirschbaum & Hellhammer, 2000), the first saliva sample provides an indication of the level of stress experienced while anticipating being taken from the classroom setting by a stranger in order to complete tests. The second sample indicates the stress level after the first few minutes of testing, while the third sample indicates the level of stress halfway through the test session.

Salimetrics saliva swabs (rimless non-sterile polypropylene-round base) were used for saliva collection. To collect saliva samples, test assistants held the swab under the tongue of the child for at least 60 seconds. If they asked children were allowed to hold the swab themselves. The majority of swabs absorbed enough saliva for analysis. Samples were analyzed at the Salimetrics Saliva Lab (Cambridge, UK), which is HTA (Human Tissue Authority) licensed. This Salimetrics Centre of Excellence uses GLP and level 2 containment protocols and equipment. The analyses are performed on an 8 fixed tips, fast wash system, robotic manipulator, two incubator units (MIOs), Tecan Sunrise and Tecan Hydroflex controlled using TecanEvoWare and TecanEvoWarePLUS software with Liebert UPS power backup. Units are enclosed in a Bigneat HEPA filter cabinet. Samples were stored at -80°C. When assaying samples were kept at 4°C on ice at all times and returned to -80°C directly after use.

Statistical analyses

Assessment of the missingness mechanism was first performed (Little, 1986) because nonrandom missingness would lead to biased complete case analysis. To further account for missing data, analyses using imputed data were repeated twice. To assess sensitivity of missing data handling, once imputed data were generated under strict assumptions concerning the distribution of data (m = 100, N = 440) and empirical range, and once under less strict assumptions (m = 100, N = 440).

To assess differences in patterns of stress reactivity in children born late preterm and their full-term peers mixed-model ANCOVAs were applied, with repeated measures for salivary cortisol levels (µg/dL) at the three different time points. 'Late preterm birth vs. full term birth' was considered as the between-subjects' factor. As covariates in the analysis were entered: time (in minutes) between the different saliva measurements and time of day at the start of the test session. Time of day was included since cortisol levels do not only fluctuate in response to stress but also follow a diurnal pattern (Van den Bergh, Van Calster, Smits, Van Huffel, & Lagae, 2008).

Results

Missing data

Based on the procedure as described by Little (1986) we were able to reject the null hypothesis of data being not missing completely at random (χ^2 = 20.78, *p* = .187), therefore complete case analysis could be applied.

Sample characteristics

Children were on average 67.13 (*SD* = 3.84) months old and a small majority was male (57.2%). Generally, average cortisol levels did not differ largely between time points (start of the session, halfway through the sessions, and at the end of the session) at which saliva collection took place (range between .08 µg/dL and .09 µg/dL) (Table 1).

Table 1. *Descriptives (means and standard deviations / percentages) of background variables (i.e. age, sex, father's educational level), late preterm birth, and cortisol assessments)*

***Descriptives* (*N* = 250)**	**Mean (*SD*) / percentage**
Age, months	67.16 (*3.85*)
Sex, male	57.8%
Educational level father (max = 6)	3.85 (*1.40*)
Cortisol level 1: beginning test session (µg/dL)	.09 (*.05*)
Cortisol level 2: halfway test session (µg/dL)	.09 (*.07*)
Cortisol level 3: end test session (µg/dL)	.08 (*.05*)
Time (min.) between collection sample 1 and sample 2	16.65 (*3.23*)
Time (min.) between collection sample 2 and sample 3	17.85 (*4.35*)
Time of day at start test session	10.53 (*1.35*)
Born late preterm	10.0%

Patterns in cortisol trajectory

A mixed design was applied to test differences between late preterm vs. full term children, differences within participants (i.e. the course of cortisol levels in the three successive measurements), and the interaction between these between- and within-subject factors.

The assumption of sphericity was violated (*Mauchly's W* = .94, *p* < .001) and the Greenhouse-Geisser coefficient exceeded .75 (*GG* = .94). The Huyn-Feldt correction (*HF* = .96) was therefore applied (Field, 2009). Of all between-subject variables and covariates, only the time of day at which the saliva sample was collected, showed a significant association with the average level of salivary cortisol (*F* (1, 245) = 8.54, *p* = .004) over the three time points. Considering the main and interaction effects within subjects, only the interaction between the trajectory of salivary cortisol levels and late preterm birth was present (*F* (1.93, 471.76) = 4.78, *p* = .010) (Table 2).

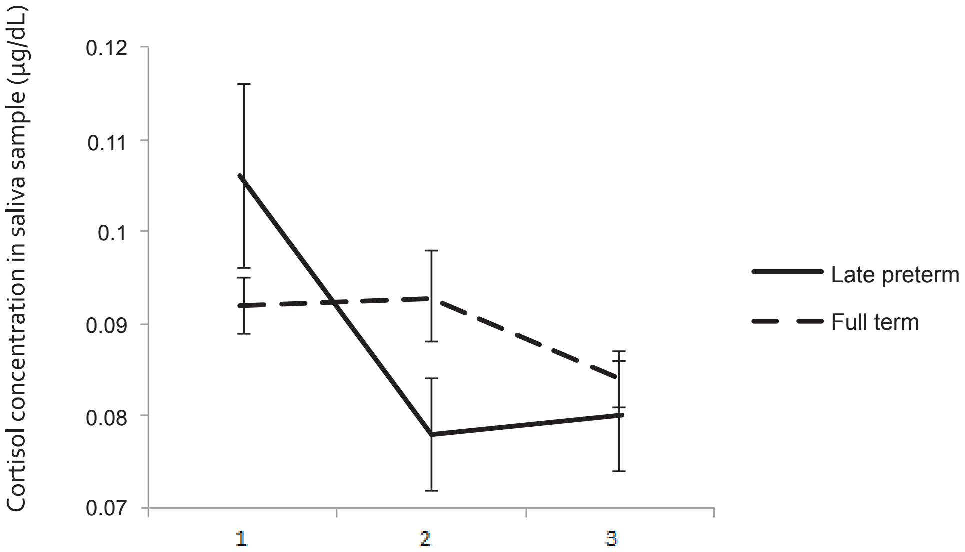
Table 2. *Results of mixed design ANCOVA with salivary cortisol levels as repeated outcome measure and late preterm and time measurements as between-subjects variables*

Predictor	***F*(*df* = 471.76)**	***p***
Between		
Preterm birth	.04	.846
Time between session 1 and 2, minutes	.37	.546
Time between session 2 and 3, minutes	.83	.365
Time of day at start test session	8.54	.004
Within		
Cortisol trajectory[1]	.41	.654
Cortisol trajectory * time of day at start test session	.33	.709
Cortisol trajectory * time between session 1 and 2	.09	.909
Cortisol trajectory * time between session 2 and 3	.34	.706
Cortisol trajectory * born late preterm	4.78	.010

1. Course of the cortisol levels found in the three successive measurements

The interaction between late preterm birth and cortisol trajectory is depicted in Figure 2, based on mean salivary cortisol levels corrected for background variables (i.e. time between the measurements and time of day).

For full term children cortisol (and thus stress-) levels remained constant over the three measurement points. For late preterm children cortisol levels dropped between the first and second measurement. Additionally, the first measurement was elevated, whereas in children with mild perinatal adversities, this was not the case. Between the second and the third point stress levels remained constant.



$N_{no\ mild\ perinatal\ adversities}$ = 173; $N_{mild\ perinatal\ adversities}$ = 77 ; $N_{full\ term}$ = 225; $N_{late\ preterm}$ = 25

Figure 2. Cortisol trajectory (µg/dL) late preterm vs. full term children

Post-hoc analysis confirmed that the significant differences found in the course of salivary cortisol between late preterm children and full term children were attributable to differences in the gradient of slopes between the first and the second measurement (F (1, 245) = 7.91, p = .005), and not to differences between the second and third measurement (F (1, 245) =1.28, p = .260).

Repetition of the analysis in imputed datasets yielded similar results (Supplementary Table 1). All results remained constant, including the significant finding regarding the interaction between late preterm and cortisol trajectory (complete cases: F (1, 242) = 4.61, p = .010, multiple imputation under strict assumptions: F (1, 432) = 6.60, p = .001), multiple imputation without strict assumptions: F (1, 432) = 8.03, p <.001).

Discussion

We expected five-year-old children born late preterm to show increased levels of stress reactivity as compared to their full term peers, and thus to show elevated levels of stress in anticipation of a possibly stressful event (i.e. testing by an unfamiliar adult). However, since late preterm children have been shown to react well to guided, structured environments (Merkelbach et al., 2018), a decrease in experienced stress when the test setting turned out to be highly structured and guided was expected in this group. On the other hand, we expected the stress levels of full term children to remain relatively constant over the course of these events since they are not expected to experience increased levels of stress reactivity. Results confirm that patterns in salivary cortisol, which were used as a proxy of experienced stress, differ between late preterm children and their full term peers (F (2, 471.76) = 4.78, p = .010). In anticipation of being tested, salivary cortisol levels were elevated in children born late preterm, but as soon as testing had started, a clear decrease took place. In full term children stress levels remained constant (F (1, 245) = 7.91, p = .005).

Children born late preterm do thus show a different neurobiological reaction pattern during stressful events, like anticipating being tested by a stranger, as compared to their peers, suggesting an elevated level of stress reactivity (Kolb & Whishaw, 2009) in this group. Although increased stress reactivity can be an asset in unpredictable situations in which (physical) danger is a considerable possibility, since it offers the opportunity to react fast (Sapolsky, 2015), in low-risk situations like an educational setting, increased stress can stand in the way of effective learning. In line with the finding of Blair, Granger and Peters Razza (2005) that stress reactivity is associated with executive function, self-regulation, and letter knowledge, we might expect that deviations in stress reactivity patterns might prevent children from absorbing information from the environment.

Although elevated at the start of the session, not during the entire session did cortisol levels remain high in children born late preterm. When placed in the testing situation, stress levels almost immediately seemed to decrease. The testing environment employed in the current study shows great similarity to the environment offered by the digital educational interventions *Living Letters* (stimulating early literacy) and *Clever Together* (stimulating early numeracy) that have been found particularly useful to support the learning of children born late preterm (Merkelbach et al., 2018). Both the test environment and these digital programs offered structure, continuous and adaptive feedback, and guidance. Possibly these features provided children born late preterm with much needed support, clarifying the test at hand. As was the case during the testing situation, we might thus expect that while interacting with *Living Letters* and/or *Clever Together* also a decrease in the level of stress experienced by late preterm children took place. The possibly stress reducing features of these programs (and the testing situation) might explain why they are very helpful for children born late preterm, while they do not sort effects for their full term peers.

These results thus show that children born late preterm experience higher levels of stress reactivity in educational settings than their full term peers. Additionally, our findings suggest that structure, continuous and adaptive feedback, and guidance could be helpful to support the learning of highly stress reactive children, such as children late preterm, since they might have stress reducing effects. However, a direct link between

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the effectivity of such features in promoting learning and stress reactivity levels has not been established yet. Additionally, more research is needed to identify which specific features of the learning environment are essential to facilitate the learning of children showing high levels of stress reactivity.

Conclusion

Five-year-olds born late preterm do show a different neurobiological reaction pattern to stressful events than their full term peers, suggesting higher levels of stress reactivity in this group. For children who were born late preterm, the prospect of being placed in an unfamiliar, unpredictable situation resulted in increased salivary cortisol levels. However, when placed in a highly structured, guiding situation, stress levels decreased almost immediately and dropped even below the cortisol levels of their full term peers. For children born full term no fluctuations in cortisol levels have been detected. These results suggest that structured and guiding intervention programs might be highly effective for late preterm children especially, since they might have stress reducing qualities that facilitate learning in this group.

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Supplementary table 1. *Results of repeated measures ANCOVA with salivary cortisol levels as outcome measure in complete cases, and in two imputed datasets*

	Complete cases analysis		**Imputation with strict assumptions**		**Imputation without strict assumptions**	
Predictor	***F(df* = 242)**	***p***	***F(df* = 242)**	***p***	***F(df* = 242)**	***p***
Cortisol trajectory	.41	.654	.34	.560	.57	.451
Cortisol trajectory * time of day at start test session	.33	.709	.28	.597	.19	.663
Cortisol trajectory * time between session 1 and 2	.09	.909	.11	.740	.08	.777
Cortisol trajectory * time between session 2 and 3	.34	.706	.33	.566	.46	.498
Cortisol trajectory * born late preterm	4.78	.010	6.60	.011	8.03	.005