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Children's cortisol response to the transition from preschool to formal schooling:
A review

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Highlights

- We reviewed ten studies reporting on child cortisol concentrations during the transition to formal schooling.
- Most studies observed that this transition coincides with an increase in cortisol concentration in both saliva and hair.
- Recovery could take as long as 3 to 6 months yet the functional significance of the rise in cortisol remains unknown.
- Important individual differences were observed, with likely predictors including child temperament and maternal anxiety.
- We provide 5 sets of methodological recommendations and guidance for future studies.

Abstract

This review examines the current state of knowledge regarding children's biological stress response during the transition from preschool to compulsory formal schooling, focusing on longitudinal studies that include repeated measures of cortisol concentrations in saliva or scalp hair. In all, eight independent studies (ten publications) were found and their results support the hypothesis that the transition from preschool to formal schooling coincides with an increase in cortisol concentration in both saliva and hair. Evidence of recovery (i.e. decrease in stress response over time) is more limited and suggests that it could take as many as 3-6 months before kindergarten children's cortisol concentration returns to baseline levels. However, important individual differences are observed. Potential predictors that have received some empirical support include child temperament (fearfulness/inhibition or surgency/extroversion) and prenatal

maternal stress or anxiety. Very few studies, however, have examined whether there are actual functional consequences of individual differences in children's cortisol response associated with this transition. Finally, current methodological limitations and avenues for future studies are discussed.

KEYWORDS: Cortisol; Stress; School transition; Kindergarten

Children's cortisol response to the transition from preschool to formal schooling: A review

1. Introduction

The first year of formal schooling is an important developmental transition experience, for both children and their families: The school environment is structured around explicit goals of instruction, typically, literacy, numeracy, and socialization, and is increasingly focused on children's academic progress, in contrast to most childcare or home environments (Hughes, 2015; Rimm-Kaufman and Pianta, 2000). Several researchers consider school entry to be a "universal social stressor" or a "normative stressor", because children enter a novel peer-group setting and face these new social and cognitive demands (Groeneveld et al., 2013; Rimm-Kaufman and Pianta, 2000; Russ et al., 2012). While such social events are generally considered positive for most children, the social, emotional and cognitive resources required for their navigation are considerable and may cause significant stress (Quas et al., 2002). If children experience stress when entering the formal schooling system, this stress should be observable through physiological indicators associated to stress reactions in humans, for example, through changes in the activity of the hypothalamic-pituitary-adrenocortical (HPA) system, a stress-sensitive neuroendocrine system, that can be indexed by cortisol concentrations in saliva, hair or blood (Gunnar and Quevedo, 2007; Lee et al., 2015; Lupien et al., 2009). Assessment of intra- and inter-individual differences in cortisol levels, which are socially and endogenously regulated (Gunnar and Quevedo, 2007; Lupien et al., 2009), has become an important method to examine which situations stimulate a stress response, under what circumstances, and the time course of that stress response (Decaro and Worthman, 2008). Longitudinal studies of cortisol levels in children entering childcare, changing rooms/classes at childcare or starting a new nursery school year (e.g. Bernard et al., 2015; Gunnar et al., 1997; Rickmeyer et al.,

2017; Tarullo et al., 2011) have noted changes in patterns of cortisol levels suggesting a higher solicitation of the HPA axis when adjusting to childcare compared to being home. However, because children undergo very rapid developmental changes during the preschool and early school years, especially in their capacity for self-regulation (Carlson et al., 2013; Zelazo and Carlson, 2012), conclusions from studies on the transitions to daycare cannot be presumed to apply to the transition to elementary school. Notably, rapid u-shaped age-related differences have been reported in children's stress reaction associated with childcare attendance during the infant, preschool and early school age: Those children most likely to show an increased physiological stress reaction (as assessed through midmorning to mid-afternoon patterns of salivary cortisol) during childcare compared to home are aged between 3 and 5 years, compared to both younger (3 to 24 mo.) and older children (6 to 9 years) (Bernard et al., 2015; Geoffroy et al., 2006; Vermeer and Van Ijzendoorn, 2006). Further, because the elementary school environment includes novel characteristics compared to childcare settings (e.g. not only new peers and teachers, but also larger classrooms, new rules, increased structure, explicit learning goals, as well as expectations for greater independence and responsibility, see Decaro and Worthman, 2008; Rimm-Kaufman and Pianta, 2000), results from childcare settings cannot be presumed to apply to school settings.

Understanding the extent to which school entry represents a stressful challenge for children is important as there is cumulative evidence that repeated exposure to stressful experiences in the years before or during the first years of schooling may be associated with negative functional outcomes in both learning and mental health (Gunnar and Quevedo, 2007; Lupien et al., 2009). For example, pregnancy stress and family instability during the preschool period were found to be linked to poorer cognitive abilities at school age (Gutteling et al., 2006). Accumulation of markers for economic disadvantage in kindergarten was associated with reduced gains in tests of math and

reading ability from kindergarten to first grade, in part through the mediating role of child socio-emotional problems (Crosnoe and Cooper, 2010). Stressful social experiences in kindergarten were negatively associated with classroom participation and academic achievement (Ladd et al., 1999). Finally, exposure to family adversity combined to indicators of physiological reactivity to stress in young children has been shown to relate to later mental health (Boyce et al., 2006; Laurent et al., 2014b). Despite all of this research, it remains to be clarified whether actual entry into the formal school system is stressful for children, for how long a stress reaction is observed, whether some children experience higher levels or more prolonged periods of stress during that transition, and whether this stress reaction or its duration has functional consequences or predictive significance. It is particularly important to clarify what is normative with regard to the pattern and duration of physiological reactivity to school entry and what is atypical and should capture our attention because school entry is a key opportunity to systematically screen, assess and support vulnerable children and their families. Indeed, developmental research has shown that individual differences in the way children experience this transition and adjust to a formal instruction environment predict their future school engagement, academic progress and psychosocial adjustment throughout the elementary school years and beyond (e.g. Archambault et al., 2013; Blair et al., 2016; Burt and Roisman, 2010; Hamre and Pianta, 2001; Kiuru et al., 2015; Lee and Bierman, 2015; Pagani et al., 2012).

This review examines the current state of knowledge regarding children's biological stress response during the transition from preschool to compulsory formal schooling (preschool to kindergarten¹, or kindergarten to first grade), focusing on

¹ Kindergarten is used here to designate a pre-primary class that is compulsory in a given country. In some countries (e.g., Netherlands), children may have spent some time in a pre-primary class on a voluntary basis before they entered compulsory kindergarten. In other countries (e.g., UK), children may start kindergarten on a part-time basis.

longitudinal studies that include repeated measures of cortisol concentrations in saliva or scalp hair either (i) before and after school entrance or (ii) at intervals after school entry. Because school systems differ across countries, the age of entry into kindergarten varies across studies (4 or 5 years) and the number of years spent in kindergarten also varies (1 or 2 years). Four questions are addressed. (1) Is there an increase in cortisol concentration that coincides with the transition from preschool to kindergarten or from kindergarten to first grade? (2) Is there evidence of recovery (i.e. decrease in stress response over time) and how long after entry is it observed? (3) Are there individual differences in the magnitude of the increase in child cortisol concentration associated with this transition or individual differences in length of recovery and what factors might account for these individual differences? (4) What are the consequences of an increase in children's cortisol concentration level associated with this transition? Finally, we address current methodological limitations and suggest avenues for future studies.

In order to address these questions we sought to identify peer-reviewed, full-length reports examining repeated measures of cortisol concentration, either from saliva or hair, during the transitions to kindergarten or first grade, in neurotypical populations (studies were excluded if they focused on children with autism spectrum disorders or if they included a combination of children from undifferentiated grade levels). An extensive search of the Web of Science, PsycINFO and Google Scholar were conducted using the following terms: [(cortisol OR stress) AND (child* OR preschool) AND (school transition OR school entry)] – excluding studies focusing on autistic spectrum disorder. Systematic examination of the reference lists of retrieved articles and search for citing articles of retrieved articles were also conducted, up to May 8, 2018. Ten studies were found, reporting on eight independent samples (see Table 1 for details about each of these studies).

Insert Table 1 about here

1.1 Is There an Increase in Cortisol Concentration That Coincides with the Transition from Preschool to Kindergarten or from Kindergarten to First Grade?

Nine out of the ten retrieved studies (seven independent samples) examined the transition to kindergarten. In these studies, cortisol concentrations were assessed before and after the transition from preschool (including different combinations of childcare and voluntary preschool arrangements) to kindergarten in the US, UK or Netherlands (Boyce et al., 1995; Decaro and Worthman, 2008; Groeneveld et al., 2013; Gutteling et al., 2005; Hall and Lindorff, 2017; Quas et al., 2002; Russ et al., 2012; Turner-Cobb et al., 2008; Yang et al., 2017). Among those studies, 8 examined change in salivary cortisol concentration as an indication of children's biological reactivity to the school transition and one examined hair cortisol concentration, as a biomarker of cumulated stress before and after the transition (for further details about meaning and validity of salivary and hair cortisol assessments, see Jessop and Turner-Cobb, 2008; Lee et al., 2015; Stalder and Kirschbaum, 2012; Stalder et al., 2016; Vanaelst et al., 2012).

Five² of these kindergarten studies, reporting on five of the seven independent samples, found an increase in cortisol concentration between pre- and post-transition measures, in saliva (Boyce et al., 1995; Decaro and Worthman, 2008; Gutteling et al., 2005; Russ et al., 2012) or hair (Groeneveld et al., 2013). Pre-transition salivary cortisol

² One study (Decaro, J.A., Worthman, C.M., 2008. Return to school accompanied by changing associations between family ecology and cortisol. *Developmental Psychobiology* 50, 183-195.) provided means and standard deviations, but did not provide significance tests for pre- to post-transition changes in SCC. We computed paired sample *t* values, using the formula: $= \frac{\Delta d}{\sqrt{s^2/n}}$, where Δd corresponds to the mean

difference between pre- and post-transition SCC, s^2 corresponds to the sample variance in SCC and n corresponds to the number of participants.

concentration (SCC) was assessed at different periods across studies, from several months to a week pre-transition. In all but one study, post-transition SCC was assessed in the first week of schooling (between the first day and one week post-transition). In every case, morning SCC (awakening or 30 min. post-awakening) was higher at post-transition compared to pre-transition. In one study (Decaro and Worthman, 2008), an increase was also found for evening SCC (7 PM). Hair cortisol concentration (HCC) was measured using 5 cm of hair, collected 2 months post-transition and separated in two specimens (Groeneveld et al., 2013): First 2 cm was used to assess cumulative stress during the 2nd and 3rd months pre-transition and last 2 cm were used to assess cumulative stress in the first 2 months post-transition. In line with results from SCC studies, HCC was also found to be higher post-transition, which suggests that children not only show an acute stress response to the school transition, as indexed through SCC, but they also experience heightened long-term cumulated stress in the first two months of schooling, as indexed through HCC. However, two of the seven independent kindergarten studies failed to find a significant increase in cortisol concentration between pre- and post-transition measures (Turner-Cobb et al., 2008; Yang et al., 2017). In those two UK studies, SCC was assessed pre-transition and post-transition in children entering kindergarten as four-year-olds, with some of them on a part-time schedule. Note also that pre-transition SCC in Turner-Cobb et al.'s (2008) study was assessed 4 months prior to transition and was found to be elevated compared with pre-transition SCC in other studies, as well as compared to 6 months post-transition SCC in the same study (see Table 2 for a summary of SCC values found in reviewed studies). Finally, in addition to those seven independent studies, two re-analyses were published. (1) A re-analysis of data from the same sample as Boyce et al. (1995) that included slightly more participants (50 vs. 39) did not replicate the original results: The very small increase in morning SCC from pre- to post-transition into kindergarten was found to be non-

significant, mostly due to higher pre-transition SCC values (Quas et al., 2002). (2) A re-analysis of data from the exact same sample as Turner-Cobb et al. (2008) replicated the findings of the original study: Using a different analytical approach, a non-significant increase in cortisol awakening-to-early-evening slope³ steepness from pre- to post-transition into kindergarten was found (Hall and Lindorff, 2017).

Insert Table 2 around here

With regard to the transition to first grade, only one study was found, in which SCC was assessed repeatedly after entry into first grade (no pre-transition assessment) (Bruce et al., 2002). SCC was assessed three times per day: 30 minutes post-awakening, in late afternoon and at bedtime. It was found that the SCC diurnal slope⁴ on the first school day was steeper than the post-transition mean SCC slope of two weekend days: morning SCC was somewhat higher on the first school day (close to significant), whereas afternoon and bedtime SCC were significantly lower compared to weekend days.

In sum, despite a small number of studies and generally small sample sizes, there is some evidence that the transition to kindergarten is associated with an increase in cortisol concentration, as measured in both saliva and scalp hair, at least in the US or Netherlands, as studies conducted in the UK did not find such an increase. The evidence is much weaker for first grade, as only one study examined cortisol concentration upon school entry, and it did not include pre-transition cortisol assessments. Some unanswered questions would need to be addressed, however, in

³ In this study, diurnal slopes were calculated by subtracting the mean awakening from the mean evening value (over 2 consecutive days).

⁴ In this study, diurnal slopes were obtained by regressing cortisol values on sampling time, using the resulting slope as an indicator of the magnitude of change in SCC over the day.

order to make the evidence stronger. For example, we do not know if there could be a possible effect of the period in the year when pre-transition measures of cortisol concentration are taken, as none of the reviewed studies collected pre-transition samples at more than one period of the year (see Methodological Issues section below).

1.2 Is There Evidence of Recovery (i.e. Decrease in Cortisol Concentration over Time) and When Is It Observed?

Only four of the reviewed independent studies measured SCC at more than one point post-transition in kindergarten, allowing the authors to test whether children recovered from the observed increase in cortisol concentration. Two of these studies did not find any recovery, after either a week (Gutteling et al., 2005) or through the first kindergarten term (Russ et al., 2012). In fact, the latter study found afternoon SCC to increase between the first week of kindergarten and the end of first term (Russ et al., 2012). This unexpected result was replicated in a third kindergarten study where SCC was found to significantly increase between pre-transition assessments and 3-months post-transition assessments (Yang et al., 2017). Recovery was nevertheless observed 6 months post-transition in that third study for midafternoon cortisol (between 2:00-4:00PM) and area under the curve of diurnal cortisol (Yang et al., 2017), and in a fourth study for both awakening and early evening cortisol (5:30PM) (Turner-Cobb et al., 2008). Interestingly, these results for kindergarten are consistent with results from studies of children in childcare: In one of the few prospective studies of patterns of cortisol concentration during the transition to a new childcare, Bernard et al. (2015) also did not observe any recovery after 2.5 months post-transition. Another study assessed post-transition SCC only once, but within a large time window (3 to 12 weeks post-transition) and found that individual differences in evening cortisol concentration were negatively associated with the number of days since kindergarten entry: Children assessed closer rather than later in time after kindergarten entry had higher evening cortisol

concentrations (Decaro and Worthman, 2008). In a final kindergarten study, HCC from a subsample of 11 children was reanalyzed in order to compare values in the first month post-transition to kindergarten to HCC in the second month post-transition: In line with previous SCC studies, no recovery was found (Groeneveld et al., 2013). Finally, in one study of transition into first grade, SCC was measured at more than one point post-transition (Bruce et al., 2002): While the SCC diurnal slope on the first day of school was steeper than the SCC diurnal slope on weekend days, it was no longer the case for the school-day SCC diurnal slope one-week post-transition into first grade, suggesting a much shorter recovery than in kindergarten.

There is thus some evidence of recovery, but it remains unclear at what rate it occurs and whether the rate of recovery varies developmentally between kindergarten and first grade. Existing studies suggest that recovery could be much faster in first grade than in kindergarten, where it could take 3-6 months instead of a few days. Repeated measurements of SCC or HCC over the year in both kindergarten and first grade would be necessary to clarify this issue. Further, it might prove relevant, for both kindergarten and first grade, to examine individual differences in rates of recovery and their possible associations with functional outcomes (e.g. school learning outcomes, peer relations, externalizing or internalizing problems, somatic complaints), in order to eventually identify children who might be more vulnerable or at risk for further adjustment difficulties.

1.3 Are There Individual Differences in the Magnitude of the Increase in Cortisol Concentration Associated with This Transition or in Recovery and What Factors Account for These Individual Differences?

Reviewed studies have found important individual differences in the magnitude of change in child cortisol concentration through the transition, as indicated by elevated variance in cortisol concentration values both pre- and post-transition (see Table 2).

Several potential predictors of these individual differences have been examined, including child characteristics (sex, temperament or social adjustment, see Bruce et al., 2002; Groeneveld et al., 2013; Hall and Lindorff, 2017; Quas et al., 2002; Russ et al., 2012; Turner-Cobb et al., 2008; Yang et al., 2017), prenatal maternal characteristics (stress, anxiety or phobia, see Gutteling et al., 2005; Russ et al., 2012), family characteristics (maternal education, age or working status, single-parent status, child birth order, quality of parent-child or family relationships, stressful life events or change in family routines, see Boyce et al., 1995; Decaro and Worthman, 2008; Groeneveld et al., 2013; Hall and Lindorff, 2017; Quas et al., 2002; Russ et al., 2012; Turner-Cobb et al., 2008; Yang et al., 2017), and childcare attendance (number of months, hours or days per week, see Decaro and Worthman, 2008; Groeneveld et al., 2013; Hall and Lindorff, 2017; Turner-Cobb et al., 2008). In short, reliable conclusions regarding the most likely predictors of individual differences in cortisol concentration increases from pre- to post-transition or in the extent of recovery are yet to come, as different studies have examined different predictors, typically using small sample size, with very few replications. Yet, in line with some childcare studies (Geoffroy et al., 2006; Tarullo et al., 2011; Vermeer and Van Ijzendoorn, 2006), two dimensions of child temperament were found to predict a greater increase in cortisol concentration or a slower recovery, in both SCC and HCC: A shy, inhibited or fearful temperament and its opposite, high surgency/extroversion. Possible moderators (e.g. intensity/quality of previous childcare experience, social isolation, maternal prenatal stress, anxiety or social phobia) and the meaning of these associations still remain to be clarified though and could vary as a function of both child characteristics and context (see for example, Tarullo et al., 2011). Prenatal maternal stress and anxiety have also been associated with higher child SCC post-transition to kindergarten in two independent studies (Gutteling et al., 2005; Russ et

al., 2012), but heterogeneity in results depending on which specific measures of stress or anxiety were used makes any firm conclusion premature.

Further, given that school entry is conceived as a challenge to which children react by increasing their production of cortisol and in contrast to studies relating childcare quality with SCC in preschool children (e.g. Lisonbee et al., 2008), it might seem paradoxical that no study so far has examined potential moderators from the school environment itself. For instance, are there characteristics of the school environment that could accentuate or attenuate cortisol concentration increase upon entry or that could help support recovery? Some studies suggest that the teacher-child relationship may be a key variable to consider. For example, in preschool settings, higher emotional support from the teachers was associated with a greater decline in children's SCC from morning to afternoon (Hatfield et al., 2013). In first grade, a more conflictual teacher-child relationship was associated with flatter cortisol profiles (less decrease over day), whereas a proximal/balanced relationship was associated with a more optimal stress regulation pattern (Ahnert et al., 2012). More generally, an important body of research provides evidence for compensatory effect of the quality of the teacher-child relationship in kindergarten or first grade for children at high risk of behavioral problems or academic failure (e.g. Brendgen et al., 2011; Griggs et al., 2009; Sabol and Pianta, 2012). Children's SCC as a function of characteristics of the school environment, notably the teacher-child relationship, need to be studied explicitly during the transition to school.

1.4 What Are the Consequences of an Increase in Children's Cortisol

Concentration Associated with the School Transition?

Reviewed studies so far have shown evidence supporting the hypothesis that the transition from preschool to formal schooling is associated with an increase in cortisol concentration for most children and that recovery is not observed within the first weeks

or months post-transition. There is also evidence for individual differences in the magnitude of this reactivity to the school transition and rate of recovery. However, it is unclear how these findings are associated with child functioning. Accordingly, examination of the academic or physical and mental health correlates or consequences of an elevated or prolonged increase in SCC or HCC for children is needed. However, very few studies have addressed this key question. Two kindergarten studies have examined how SCC was associated with children's immune system function whereas two other studies examined associations with parent- or teacher-reports of kindergarten and social adjustment. One study found that increases in morning SCC from pre- to post-transition were associated with immune system response one-week post-transition, namely an association was found with an increase in number of B cells and less effective B cell-mediated antibody production (Boyce et al., 1995). Another study found that higher early evening cortisol 2 weeks post-transition predicted less frequent upper respiratory tract infection (URIs) during the first 6 months of schooling (Turner-Cobb et al., 2011). This latter study also found that a more abrupt diurnal slope of SCC at post-transition, combined with social isolation in children, predicted more severe URIs in the first 6 months of schooling. Higher post- but not pre-transition evening SCC was found to be correlated with poorer academic functioning, although this association was attenuated and became nonsignificant once dysfunctional parent-child interactions were added to the model (Decaro and Worthman, 2008). Finally, changes in adaptive behaviors over the first 6 months of kindergarten, as reported by teachers, were not associated with child post-transition SCC (Turner-Cobb et al., 2008).

As Zalewski and colleagues (Zalewski et al., 2015) pointed out, without functional indicators to corroborate the meaning of the cortisol levels observed, it is difficult to know to what extent specific levels of morning cortisol or diurnal slopes can be interpreted as indicators of physiological reactivity to the school environment, especially since the

profiles of highly elevated or very low morning cortisol concentration are both considered adaptive to environmental stressors but may represent potentially dysregulated forms of HPA-axis functioning if maintained over the long run. Indeed, research with preschoolers, children and adolescents have found associations between both high and low morning cortisol concentration and different indicators of mental health or psychosocial adjustment (e.g. Laurent et al., 2015; Laurent et al., 2014b; Lengua et al., 2013; Ruttle et al., 2011; Saridjan et al., 2014; Shirtcliff and Essex, 2008). However, it remains to be tested whether an increase in cortisol concentration in either saliva or scalp hair upon school entry or slow recovery is associated with any physical, mental health or psychosocial adjustment correlates or outcomes and under what conditions.

2. Methodological Limitations and Future Directions

Our review also revealed some weaknesses and great heterogeneity in methodology across studies that introduced uncertainty in the interpretation of results. Accordingly, how should we ideally conduct studies aimed at clarifying the magnitude and duration of the stress response during the transition to school, as well as for whom this stress response is maladaptive? We first wish to emphasize that the field would largely benefit from well-powered studies that would allow for reliable examination of small to moderate size intra- or inter-individual differences, as well as exploration of patterns or profiles of cortisol responses and their functional correlates. Several authors provide sound guidance to help researchers determine the sample size needed to test such complex developmental hypotheses (e.g. Fritz and MacKinnon, 2007; Hedeker et al., 1999; Lenth, 2001; Wolf et al., 2013). Apart from sample size, however, other important methodological issues need to be addressed in order to produce interpretable results that reduce alternative interpretations.

The following section addresses five questions specific to the method and timing of assessment of cortisol concentrations during the transition into kindergarten or first grade, which lead to specific recommendations that should increase the interpretability of results. (1) Should saliva (acute stress) or hair (chronic stress) be collected in order to assess changes in cortisol concentrations during the transition to school? (2) At what time of the year should pre-transition measure(s) of cortisol concentration be collected in order to obtain an adequate baseline measure? (3) When is the best period of the year for taking recovery measure(s)? If saliva is used: (4) What are the best days of the week to collect it and for how many consecutive days should it be collected? (5) What time(s) of the day is/are best for saliva collection?

2.1 Should Saliva or Hair Be Collected in Order to Assess Changes in Cortisol Concentrations during the Transition into School?

All reviewed studies assessed cortisol concentrations in saliva, with only one exception (Groeneveld et al., 2013). Salivary cortisol concentration measures are well known and frequently used. SCC is considered a good biomarker of acute stress: It is sensitive to rapid changes in unbound cortisol concentration in serum and plasma, which represent the biologically active hormone fraction (Lee et al., 2015). Because it is a non-invasive procedure, it is well suited for repeated measurements and use in children. However, disadvantages of this approach include its diurnal variation, which can make interpretation and comparisons of results across studies challenging. For this reason, most reviewed studies have assessed SCC repeatedly across the day, creating a large amount of data. Varied analytical strategies have been used to combine the data into interpretable scores, adding to the challenges of interpretation and comparison of results across studies. Nevertheless, with adequate sample size and using analytical strategies that allow for examination of both intra- and inter-individual differences over time (e.g. latent change score modeling, McArdle, 2009; McArdle and Hamagami, 2001), repeated

assessment of SCC within days and across weeks or months could allow the identification of patterns of cortisol regulation that could be more informative than overall cortisol concentration levels.

Hair cortisol concentration measures have been introduced more recently. HCC is used as a biomarker of the accumulation of stress over time (chronic stress) (Lee et al., 2015), and its validity is increasingly recognized (Russell et al., 2015; Stalder and Kirschbaum, 2012). Because cortisol accumulates in scalp hair as it grows, and because scalp hair grows at a rate of approximately 1 cm/month, HCC provides a simple retrospective measure of cumulated cortisol production. Three potential disadvantages related to the study of school transition using HCC should be mentioned, however. First, it can be difficult to reliably relate HCC values to specific events, such as school entry, as hair growth rate can vary across people. For example, the rate of hair growth in adults has been shown to be slower in people of African descent and faster in people of Asian descent, especially women, compared to people of European descent (Loussouarn et al., 2005). Second, there is an elevated risk of missing values due to very short hair (8% of participants in Groeneveld et al., 2013's study), an attribute likely to be correlated to other (potentially unmeasured) child characteristics. Third, as cortisol leeches from the hair over time (Stalder and Kirschbaum, 2012), hair would need to be sampled twice so that the post-school entry sample is the same distance from the scalp as the preschool entry sample.

Thus, the method of choice under ideal circumstances should reflect the research questions. If one is interested in children's cortisol response to school entry, how this response is regulated throughout the day, and how it evolves over weeks, then repeated measures of SCC would be well suited. On the other hand, if one aims to examine whether participation in the school environment produces an allostatic load (chronic stress) over months for some children, and how this is associated with future physical or

mental health problems, then HCC might be a better choice. For both methods, validity and interpretability of results will be highly dependent on efforts to overcome the methodological challenges inherent to each (for detailed methodological recommendations, see Jessop and Turner-Cobb, 2008; Rotenberg and McGrath, 2014; Russell et al., 2015; Stalder and Kirschbaum, 2012; Stalder et al., 2016).

2.2 At What Time of the Year Should Pre-Transition Measure(s) of Cortisol Concentration Be Collected in order to Obtain an Adequate Baseline Measure?

Reviewed studies collected cortisol concentration measures at some point between January and August in the same calendar year before school entry (i.e. from a week before school entry to several months), sometimes on weekend days, with none of them collecting samples in more than one period, and half of them collecting pre-transition measures during the summer. Given actual data, we cannot determine that it is the actual transition to school that is associated with an increase in cortisol concentration, or whether it is confounded by either the transition in routines from the summer (or weekend) to the school days or by seasonal variations in cortisol concentrations. Only two studies compared cortisol concentrations on weekdays during winter or spring before school entry to post-transition SCC measures: one study did not find any increase in SCC (Turner-Cobb et al., 2008), mostly because of high pre-transition SCC values (see Table 1), and the other study did not explicitly test the difference between pre- and post-transition measures (Decaro and Worthman, 2008).

In order to rule out these possible routine and seasonal confounds, we need pre-transition measures that will allow one to account for: (1) Variations in daily family or child routines, such as during family vacations or weekend days (for example, very different awakening time) compared to weekdays (Russ et al., 2012; Stalder et al., 2016) or whether a child attends childcare or preschool or stays home; (2) Seasonal variations in cortisol secretion (cf. Arsenault-Lapierre et al., 2010), where longer photoperiods are

associated with higher basal cortisol secretion; (3) Child anticipatory stress nearing the time of school entry (Rickmeyer et al., 2017), although it could be very difficult to determine when anticipation/apprehension of the transition really begins, as this may vary considerably across families.

To address these issues, multiple baseline measures would provide a valuable contribution to the literature, for example, including one collection on weekdays about 3 months before school entry and another one about 2 weeks before (i.e. at the end of May or early June and another in mid-August for school systems starting in late August/early September). Three months before school entry should be long enough before the transition, so that child anticipatory stress should not be an issue. Two weeks before school entry should be very similar to post-transition measures with regard to seasonal variations. The issue of family vacation or variations in child daily schedule might be more difficult to rule out, although it would be highly unlikely that families would be on vacation at both time points. Controlling for childcare or preschool attendance and child schedule on sampling days would also be necessary in order to examine how daily routines or participation in child group activities might be possible confounds. Finally, because the school calendars, as well as other characteristics of the schooling system, may differ across countries, adaptations of these recommendations may be needed. Indeed, details about specific characteristics of the school system should be systematically reported in order to facilitate comparisons across studies.

2.3 When Is the Best Period of the Year for Taking Recovery Measure(s)?

With one exception, reviewed studies have taken only one “recovery” measure. Two kindergarten studies did not observe any recovery either a week post-transition (Gutteling et al., 2005) or as late as the end of the first term (Russ et al., 2012; Yang et al., 2017), but two studies (Turner-Cobb et al., 2008; Yang et al., 2017) found some evidence of recovery: SCC 6 months post-transition was found to be significantly lower

compared to 2-weeks or 3-months post-transition. In first grade, the only study that we found reported recovery on the fifth day of schooling, where the SCC had become similar to weekend days (Bruce et al., 2002). Any firm conclusion about the timing of recovery would be premature, however, because of the very small number of studies and because sample differences are confounded with timing differences in recovery measures.

In order to clarify the trajectory leading to recovery, future studies will need to include measures of post-transition cortisol concentration at intervals close enough in time during the early school year to capture any quick recovery, but also for a long enough period for recovery to be observed in most children, while taking seasonal variations of stress hormones into account in the analysis of results. Further, because one preschool study found patterns of SCC change over the year (from fall to spring) that were associated with temperament and social experience (Tarullo et al., 2011), repeated cortisol assessments across the entire kindergarten school year might provide valuable information about individual differences in social adjustment. Thus, based on observations from previous studies that recovery (1) is expected to take at least between 3 and 6 months in kindergarten for most children, but (2) could be much faster in first grade, and given that (3) no longitudinal study examined change in the SCC or HCC over two consecutive school years in the transition to schooling, it is suggested that cortisol concentration be assessed repeatedly during the year that follows kindergarten entry. For example, an SCC assessment plan might look as follows: after a first salivary cortisol measure during the first week of kindergarten that would assess the reactivity to the kindergarten transition, the next measures could be taken once a month for 4-5 months, in order to assess the pace of recovery in kindergarten, followed by measures toward the end of the kindergarten year, just before entry into first grade, then during the first and second weeks, and after a month post-transition to first grade, in order to

assess reactivity to the transition to first grade and potential recovery. In any case, holiday seasons should be avoided, because of a possible confound with the anticipation of the holiday. Such a repeated assessment of cortisol over the first two years of schooling would be a valuable contribution to the literature as it would not only clarify the issue of normative timing of recovery, but also allow for the examination of potential individual differences in rates of recovery (see Bruce et al., 2002; or Tarullo et al., 2011, for examples of individual differences in recovery or SCC change over the school or preschool year).

2.4 What Are the Best Days of the Week to Collect Saliva and for How Many Consecutive Days Should Saliva Be Collected?

Reviewed studies also varied with regard to the days of the week and number of days for which they collected saliva. With regard to pre-transition measures, only one study with pre- and post-transition SCC measures assessed pre-transition SCC on a weekend day (Gutteling et al., 2005). All others assessed SCC on weekdays. In order to facilitate interpretation of the results, it is preferable to avoid weekend days, both pre- and post-transition, because daily routines may be too different from weekdays (see also research on working adults, e.g. Kim et al., 2010).

With regard to post-transition measures, Gutteling et al. (2005) obtained their saliva samples on Mondays. Others do not specify on what day they obtained their measures, but some collected saliva samples on several consecutive school days (2 to 7 consecutive days, skipping weekends). Russ et al (2012) report a mean Cronbach alpha of .51 for day-to-day stability (3 to 5 consecutive days, range = .20-.66). In order to avoid confounding with other sources of stress that might occur on weekends, saliva collection should be made on school days that are preceded by another school day: morning SCC would then reflect both the experiences from the previous school day and the anticipation of the upcoming school day (Stalder et al., 2016). With regard to the number

of consecutive days, it might be preferable to invest in multiple baselines and multiple recovery measures instead of multiple consecutive days (see Stalder et al., 2016). Other relevant contributions could be systematic studies designed to identify the most sensitive school day with regard to reactivity to school entry and rate of recovery, or the most cost-effective number of days for saliva collection.

2.5 What Time(s) of Day Is Best for Saliva Collection?

To address this question we note that available studies on school transition assessed SCC upon awakening (4 studies), 30 minutes post-awakening (3 studies), just before lunch (1 study), upon return from school or just before supper (4 studies), in the evening (1 study), or within 30 minutes of bedtime (3 studies) (see Table 1).

Most of the significant changes associated with the school transition were observed in morning SCC (either upon awakening – 2/4 studies, or 30 minutes post-awakening – 2/3 studies). Other significant results were found with regard to SCC assessed somewhere between late afternoon and bedtime (4/5 studies), though the heterogeneity of methods used and observed results preclude any firm conclusion to be drawn. For example, late afternoon SCC was found to increase between the first week post-transition and the end of the first term (Russ et al., 2012). Inhibited children of mothers diagnosed with social phobia during pregnancy had higher late afternoon cortisol (mean of pre-, post-transition, and recovery scores) and a greater increase in late PM SCC from pre- to post-transition if they were first born (Russ et al., 2012). Surgency/extroversion predicted higher early evening SCC 2 weeks post-transition (controlling for pre-transition SCC) (Turner-Cobb et al., 2008), which in turn was associated with fewer URIs in the first 6 months of schooling (Turner-Cobb et al., 2011). Post-transition evening SCC (but neither pre-transition nor morning SCC) was found to be correlated to poorer academic functioning, less parental warmth and more dysfunctional parent-child interactions (Decaro and Worthman, 2008). Finally, bedtime

SCC was observed to be higher on school days (first week of schooling and end of first term) compared to pre-transition for children of mothers with prenatal diagnostic of social phobia as well as for first-borns (Russ et al., 2012).

In a few cases, studies examined daily changes in SCC, for example, computing indicators such as diurnal slopes or a cortisol awakening response (CAR). The common feature of these indicators is to quantify the dynamic changes in SCC values over some period of time (i.e. from morning to evening in the case of diurnal slopes, or for the first 30 to 60 minutes post-awakening in the case of CAR) (see Miller et al., 2016; and Stalder et al., 2016 for typical diurnal profiles or a detailed description of CAR, respectively). Steeper declining post-transition diurnal slopes compared to pre-transition slopes were found in two out of three independent studies (Bruce et al., 2002; Gutteling et al., 2005; but see Turner-Cobb et al., 2008 and Hall & Lindorff, 2017 for diverging results). One other study found higher CAR values post-transition, reflecting higher increases in SCC in the first 30 minutes post-awakening, compared to pre-transition values (Decaro and Worthman, 2008). These results have been interpreted as reflecting a more challenged stress system in children entering kindergarten, compared to their pre-transition daily routine. Interpretation of change scores is not without its challenges, however. Other authors have argued that steeper diurnal slopes may mean better stress regulation or coping, and, conversely, that flat slopes may mean that there is less downregulation of SCC from morning to evening (e.g. Ahnert et al., 2012). Most likely, a moderate elevation of morning levels of SCC in the first 30 to 60 minutes post-awakening, combined with a moderate declining slope of cortisol during the day may reflect a less challenged stress system. Deviations from this normal diurnal cycle (see Miller et al., 2016 for an overview of typical diurnal cycles) have been associated with symptoms of mental health problems (e.g. Kudielka and Wüst, 2010; Laurent et al., 2014a; Laurent et al., 2015; Laurent et al., 2014b; Lengua et al., 2013; Ruttle et al.,

2011; Saridjan et al., 2014; Shirtcliff and Essex, 2008). Thus, whether an increase or decrease in the slope would be seen as evidence of adaptation to schooling would depend on whether the children displayed a flattened or steep slope before their first days of school, which may vary among participants, notably as a function of their exposure to adversity, or out-of-home care. This last point suggests that future studies need to adopt analytical strategies that will go beyond simple tests of pre- to post-transition mean differences in SCC (variable-oriented approaches) and will examine individual profiles of change in SCC over time (person-centered approaches). Indeed, larger sample sizes than those typically found in previous studies would then be needed. Lastly, regarding CAR, because SCC increases very rapidly in the first 30 minutes post-awakening in prepubertal children, the reliability of awakening values could be an issue and requires very careful planning of data collection (see Stalder et al., 2016 for detailed recommendations).

Finally, in contrast to childcare studies, none of the reviewed studies collected saliva samples when children were in school. In order to clarify whether it is the transition to formal schooling *per se* that is associated with elevated SCC, it might be relevant to examine pre- and post-schooling cortisol concentration from saliva samples collected in the child's out-of-home social setting, i.e., with childcare providers and other children present for pre-transition samples, and with teachers and schoolmates present for post-transition samples. It would then be possible to examine the extent to which children's levels of SCC in a formal school setting are consistent with preschoolers' levels in a social out-of-home setting such as childcare.

In short, available studies offer some evidence that an assessment of morning cortisol is sensitive to the transition to kindergarten, but the evidence is less robust with regard to the sensitivity to school entry of other daily assessments. Nevertheless, mid to late afternoon or early evening SCC measures were found to be informative in four out

of five studies that measured them, and evening or bedtime SCC measures were also informative in two out of three studies that measured them. Further, the SCC diurnal slope or CAR proved informative in three studies. We thus conclude that future studies should consider assessing SCC *at least* twice during the day: Once in the morning (either upon awakening or 30 minutes post-awakening or both), and once in mid to late afternoon or within 30 minutes of bedtime. Collection of saliva samples while children are in out-of-home settings (e.g. in childcare and in school) might also prove a useful contribution. In each case, careful consideration and recording of previous and ongoing day activities (exam days, atypical social activities, stressful events, or intensive physical activities, see for example Budde et al., 2010), would be in order to avoid confounding effects (see also Stalder et al., 2016, for detailed methodological recommendations about saliva collection and content of collection diaries).

3. Conclusion

Studies on children's response to the transition from preschool to formal schooling are consistent with those from childcare and preschool; They confirm that increase in cortisol concentration in saliva or scalp hair is observed in most children. However, much research remains to be done to fully understand at what rate children recover from this increase over time, what factors predict the important individual differences that are observed and, most importantly, what may be the functional significance of such an increase, especially when it is maintained over time (lack of recovery). We believe that three main issues need to be addressed in future studies of the cortisol response in children entering school.

The first issue is the need for appropriate baseline and recovery assessments. In order to clarify what normative cortisol responses to the school transition and normative recovery rates are, and because the timing of entry into the school system in most cases

cannot be manipulated experimentally, it will be essential to repeatedly assess cortisol concentration both *before school entry*, in order to get a valid picture of baseline cortisol concentration in children, as well as *after school entry*, in order to clarify patterns and rates of recovery. Ideally, establishing systematic comparisons across countries and school systems that vary on three key variables: Age of entry (compulsory schooling starts at different ages in different countries), schedule (school entry most often occurs in late August or early September, but other schedules exist), and intensity (e.g. number of hours per day or per week spent in kindergarten) would also be useful, as those variables might be associated with different patterns of cortisol responses or recovery rates.

The second issue that needs to be addressed in future studies is the contribution of school or family factors that could accentuate or attenuate children's cortisol response to the school transition and slow down or accelerate recovery rates. Apart from further examination of children's individual differences such as temperament that might contribute to differences in their cortisol response, a better understanding of how the school and family environments may best support children during this challenging transition would be highly useful in order to develop or encourage school or community initiatives that would be tailored to children's needs.

Finally, the third issue that should receive greater attention is the examination of both the short- and long-term functional significance of children's cortisol response to the school transition and recovery rate. A stress response is not necessarily negative (e.g. Lupien et al., 2009): it is a physiological response that may actually support the adaptation to environmental demands. It is thus very important to identify at what level or when children's cortisol response to school entry becomes dysfunctional or a threat to adjustment and healthy development.

In sum, a better understanding of what a normative cortisol response to the transition to formal schooling is, of the functional significance of an atypical response, and of which children are most likely to experience such an atypical response should help guide prevention, education and social policies aimed at supporting optimal and healthy development for all children.

Conflict of Interest

The authors,

- Sophie Parent, School of Psychoeducation, University of Montreal;
- Sonia Lupien, Psychiatry Department, University of Montreal;
- Catherine Herba, Psychology Department, University of Quebec in Montreal;
- Véronique Dupéré, School of Psychoeducation, University of Montreal;
- Megan Gunnar, Institute of Child Development, University of Minnesota;
- and Jean R. Séguin, Psychiatry Department, University of Montreal

have no conflict of interests to disclose.

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Table 1. Reviewed studies, their method, and main findings.

Studies	Country	n	Transition ^a (mean age)	Cortisol measure ^b	Measuring Times			Main Results			
					Pre- Transition	Post- Transition	Recovery	Transition effects	Recovery effects	Predictors	Consequences
Boyce et al., 1995	USA	39	K (5 yrs)	SCC (awakening)	1 week before	1 week after		Higher SCC after transition	n/a	Maternal education	Rise in SCC associated with immune response
Bruce et al., 2002	USA	35	G1 (6.8 yrs)	SCC (awakening/ 4PM/ bedtime)		1st day of school	5th day of school/ WE 1 month after	Steeper diurnal slope on 1st day vs WE	Recovery	Surgency/ Shyness	
Decaro & Worthman, 2008	USA	28	K (5.6 yrs)	SCC (CAR/ evening, 7PM) (7 days in a row)	4 to 8 mo. before	3-12 weeks after (mean 7.4 weeks)		AM and PM SCC higher after transition	PM SCC increase with time	Working mothers/ Dysfunctional parent-child interactions	SCC at 7PM POST- transition negatively associated with school functioning
Groeneveld et al., 2013	Netherlands	42	K (4.2 yrs)	HCC	2 mo. before	2 mo. after		HCC higher after transition	No recovery	Fearful children/ Less childcare experience	

Table 1. Continued.

Studies	State, Country	n	Transition ^a (mean age)	Cortisol measure ^b	Measuring Times				Main Results		
					Pre- Transition	Post- Transition	Recovery	Transition effects	Recovery effects	Predictors	Consequences
Gutteling et al., 2005	Nether- lands	29	K2 (5 yrs)	SCC (every 3 hours between 8:00AM and 8:00PM)	WE, months before	1st day of school (wakening/ right after lunch/ after school/ bedtime)	1 week after	Morning SCC higher and steeper slope after transition	No recovery	Prenatal maternal morning SCC/ Fear of a handicapped child	
Hall & Lindorff, 2017 ^c	UK	105	K (4 yrs)	SCC (7:00 AM and 5:30 PM) (over 2 days)	4 to 6 mo. before	End of 2nd week (2 consec. days)	6 mo. after (2 consec. days)	Not significant	Recovery	Less childcare/ Child sex/ Parents not living together/ Younger parents/ Less educated parents	Diurnal slopes closer to zero pre- and post- transition associated with less effortful control
Quas et al. 2002 ^d	USA	50	K (5.1 yrs)	SCC (awakening)	1 week before	1 week after		Not significant	n/a	Interaction between change in routine and preschool experience/ Parents predict an easy transition	

Table 1. Continued.

Studies	State, Country	n	Transition ^a (mean age)	Cortisol measure ^b	Measuring Times				Main Results		
					Pre-Transition	Post-Transition	Recovery	Transition effects	Recovery effects	Predictors	Consequences
Russ et al., 2012	UK	112	K - 4 to 5 yrs	SCC (30 min. after awakening/ 4PM/ 30 min. before bedtime) (3 consec. typical days)	1 mo. before	1 week after (5 consec. school days)	End of first term (4 consec. school days)	Morning and afternoon SCC higher after transition	No recovery	Mothers with prenatal social phobia Behavioral inhibition/ 1stborn/ Child sex	
Turner-Cobb et al., 2008	UK	105	K - 4 yrs	SCC (7:00AM/ 5:30PM) (2 consec. days)	4 to 6 mo. before	End of 2nd week after (2 consec. days)	6 mo. after (2 consec. days)	Not significant	Recovery	Surgency/ Effortful control/ Interaction of social isolation and surgency	
Yang et al., 2017	UK	59	K - 4 yrs	SCC (Early Morning/ Midmorning/ Mid-Afternoon/ Early Evening)	1-2 mo. before (variable days)	5th day of school	3 mo. after/ 6 mo. after	Not significant	No recovery 3 mo. post-transition/ Recovery 6 mo. post-transition		

Notes:

^a K = Kindergarten; K2 = 2nd yr of kindergarten; G1 = Grade 1^b SCC = Salivary cortisol concentration; HCC = Scalp Hair cortisol concentration^c Re-analysis of data from the Turner-Cobb et al. (2008) study^d Includes the participants from the Boyce et al. (1995) study

Table 2. Descriptive statistics (mean and standard deviation) for salivary cortisol concentration assessed pre- and post-transition to kindergarten or first grade as a function of time of day.

Time of day	Study	n	Salivary cortisol concentration ($\mu\text{g}/\text{dL}$)			
			Pre-transition	Post-transition	Post-transition	Post-transition
			Mean (SD)	1 Mean (SD)	2 Mean (SD)	3 Mean (SD)
Awakening	Boyce et al. (1995)	39	.39 (.28)	.49 (.36)		
	DeCaro & Worthman (2008)	28	.34 (.10)	.38 (.12)		
	Gutteling et al. (2005)	13-24	.53 (.15)	.67 (.23)	.69 (.19)	
	Quas et al. (2002) ^a	50	.46 (.29)	.48 (.25)		
	Turner-Cobb et al. (2008)	105	.78 (.48)	.96 (.64)	.24 (.16)	
Pre-breakfast	Yang et al. (2017)	43-49	.46 (.24)	.42 (.22)	.58 (.26)	.57 (.34)
30 min. post-awakening	Bruce et al. (2002)	35	.59 (.19) ^b	.71 (.38)	.63 (.26)	
	DeCaro & Worthman (2008)	28	.45 (.12)	.56 (.23)		
	Russ et al. (2012)					
	Group SP ^c	30	.29 (.15)	.32 (.10)	.34 (.15)	
	Group C ^c	35	.34 (.16)	.35 (.15)	.33 (.12)	
	Group GAD ^c	23	.28 (.14)	.32 (.14)	.30 (.09)	
Midmorning	Yang et al. (2017)	49-56	.22 (.14)	.21 (.12)	.22 (.16)	.17 (.09)
Before lunch	Gutteling et al. (2005)	13-23	.30 (.10)	.29 (.07)	.24 (.09)	

Table 2. Continued.

Time of day	Study	n	Salivary cortisol concentration ($\mu\text{g/dL}$)			
			Pre-transition	Post-transition	Post-transition	Post-transition
			Mean (SD)	1 Mean (SD)	2 Mean (SD)	3 Mean (SD)
Mid PM	Yang et al. (2017)	48-58	.13 (.08)	.15 (.11)	.20 (.15)	.16 (.06)
Late PM	Bruce et al. (2002)	35	.21 (.08)	.14 (.07)	.19 (.08)	
	Gutteling et al. (2005)	14-27	.15 (.05)	.24 (.08)	.20 (.09)	
	Russ et al. (2012)					
	Group SP ^c	30	.09 (.06)	.09 (.08)	.09 (.05)	
	Group C ^c	35	.07 (.06)	.09 (.09)	.08 (.05)	
	Group GAD ^c	23	.07 (.05)	.07 (.03)	.09 (.04)	
Early evening	Turner-Cobb (2008)	105	.11 (.11)	.13 (.15)	.04 (.03)	
	Yang et al. (2017)	44-48	.08 (.06)	.09 (.08)	.11 (.08)	.10 (.07)
Evening	DeCaro & Worthman (2008)	28	.06 (.03)	.09 (.05)		
Bedtime	Bruce et al. (2002)	35	.09 (.05) ^d	.05 (.05)	.06 (.04)	
	Gutteling et al. (2005)	12-25	.08 (.04)	.08 (.04)	.08 (.04)	
	Russ et al. (2012)					
	Group SP ^c	30	.03 (.03)	.06 (.05)	.06 (.05)	
	Group C ^c	35	.06 (.07)	.05 (.05)	.05 (.06)	
	Group GAD ^c	23	.06 (.08)	.04 (.02)	.04 (.02)	

Notes:

^a Includes participants from Boyce et al. (1995)^b Saliva collected on two weekend days post-transition^c SP: Social Phobia; C: Control (no-diagnostic); GAD: Generalized Anxiety Disorder^d Measure collected on two weekend days post-transition.