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Salivary cortisol levels are associated with resource control in a competitive situation in 19 month-old boys

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A B S T R A C T

Glucocorticoids (GCs) have been related to social rank in many studies across species, a particular rank giving rise to a particular stress-related physiological profile. Our aim was to examine the hypothesis that GCs levels in toddlers would be related to social dominance in a competitive resource situation. Subjects were 376 toddlers from the Quebec Newborn Twin Study. At 19 months of age, each subject was exposed to 2 unfamiliar situations known to be moderately stressful at that age. Saliva was collected before and after the unfamiliar situations, to assess pre-test and reactive cortisol. Then the toddler reaction to a competitive situation for a toy with an unfamiliar peer was assessed and we measured the proportion of time the child controlled the resource. In girls, no association between cortisol levels and the proportion of time the child got the toy was found. On the other hand, in boys, increased cortisol levels before the unfamiliar situation were significantly related to a decreased proportion of time they got the toy in the competitive situation ($r_{174} = -0.17$, $P = 0.02$). These results show that even in toddlers with limited social experience, association between GCs levels and social dominance can be found, an association that is specific to boys.

Introduction

Many studies have shown that in order to achieve high social rank in a group, children use various behavioral strategies including aggression, cooperation, affiliation, depending on the context and stage of development (Hawley, 1999, 2002). Accordingly, the contemporary concept of social dominance is centered on resource control, and defined in terms of actual success at acquiring and utilizing resources (Charlesworth and La Freniere, 1983; Pellegrini, 2008). Resource control contests among unfamiliar peers have been observed in children as young as 19-months of age (Plusquellec et al., 2007).

Studies using competitive situations in preschoolers have shown that variability in resource control between individuals is independent from the child sex even though the strategies selected to control resources may vary as a function of sex (Charlesworth, 1996). For examples, girls show more verbal behavior than boys, and boys show more physical behavior than girls to control resources in preschoolers (Charlesworth and Dzur, 1987). Another interesting related point deals with emotional regulation. At this stage of development, children should use a variety of adaptative strategies (aggression, cooperation) to be successful in acquiring resource (Hawley, 2002). Studies show that children who have developed the ability to manage their own emotional reactions, either on their own or with the help of particular caregiving practices, are less likely to develop maladjusted strategy, such as disruptive behaviors (Rubin et al., 1995). We could hypothesize that this adaptative process would be limited if children are less able to regulate their emotions. Other studies suggest that boys have a lower level of emotion regulation than girls at age 2 (Hill et al., 2006), and even in infancy (Weinberg et al., 1999). Consequently, any study looking at resource control in toddlers must take into account these potential sex differences in emotional regulation.

Interestingly, both resource control and emotional regulation have been shown to be associated with variations in stress hormone levels in primates and humans (Sapolsky, 2005; Stansbury and Gunnar, 1994; Zimmermann and Stansbury, 2004). Glucocorticoids (GCs),

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which are hormones released by the hypothalamic–pituitary–adrenal (HPA) axis in response to a stressful situation, have been related to social rank in many studies across species. What most of the results show is that social rank is a strong predictor of GCs levels in both animals and humans (Creel, 2001; Hellhammer et al., 1997; Sapolsky et al., 1997). The results pointing to the association between social rank and GCs led to the formulation of two main hypotheses. The first hypothesis, the "stress of domination" hypothesis (Creel et al., 1996; Mooring et al., 2006), posits higher GCs in dominant individuals in comparison to their subordinates, because dominants must fight more than subordinates to maintain their position. In contrast, the "subordination stress" hypothesis (Blanchard et al., 1993; Shively, 1998) may also be observed and explained by the fact that subordinates are subjected to greater harassment and less control than dominants, and thus experience elevated GCs secretion. These two hypotheses suggest that social stress may be experienced by both high- and low-ranking individuals, and may vary as a function of behavioral traits associated with high and low rank (Ray and Sapolsky, 1992; Virgin and Sapolsky, 1997), as well as with the stability of the social hierarchy (Creel, 2001; Sapolsky, 2005). In stable hierarchy, subordinates are more psychologically and physically harassed, they lack social control and predictability, and need to work harder to obtain resources than dominants. On the other hand, in unstable hierarchies, or when a group is living a major hierarchical reorganization, dominant individuals become at the center of the social tensions, and thus experience more stress than subordinates. This variation in the levels of GCs as a function of the stability in hierarchy has recently been observed in a large sample of Scottish adolescents. While the scholastic hierarchy was inversely related with GCs, more unstable hierarchy, such as sport hierarchy was associated with GCs in the opposite way (West et al., 2010).

There is evidence that the association between cortisol and social dominance may occur early in life. In castrated male domestic pigs, increasing cortisol secretion following dyadic encounters between two unfamiliar individuals (no previous social experience) was significantly related to the number of observed submissive behaviors (Fernandez et al., 1994). In female and male rhesus infant (6–9.5 months of age) with no previous social experience, significantly lower baseline cortisol levels between two infants, but not reactive cortisol levels, were good predictors of the ability to dominate in dyadic situation (Golub et al., 1979). In juvenile tufted capuchins, high cortisol reactivity to isolation has been associated with lower observed levels of play with other animals, and ratings of high submissiveness throughout development (Byrne and Suomi, 2002).

Altogether these results suggest that the association between GCs and social dominance could be observed very early in development. These results indicate that lower levels of cortisol would be related to a higher dominance ability when individuals have low level of social experiences. However, no research has yet examined this point in humans. The main goal of the present study was thus to determine whether lower GCs levels in 19-month old toddlers are associated with higher social dominance in a competitive resource situation. Sex differences may emerge as a function of emotional regulation, boys having greater difficulty than girls in maintaining affective regulation as early as 6 months of age (Weinberg et al., 1999) and social maturation in childhood, girls acquiring earlier adaptive skills in social situations (Keenan and Shaw, 1997). As a consequence, we also assessed potential sex differences on the association between GCs and social dominance in this cohort of young children.

Methods and materials

Participants

Participants were families of twins from the Québec Newborn Twin Study recruited between April 1995 and December 1998 in the greater Montréal area (Canada) to participate in a longitudinal study. A total of 989 families were contacted, of which 672 agreed to participate (68%). Twins were first seen when they were 6 months of age and then prospectively assessed on a variety of child and family characteristics. After informed consent was obtained from the parents, saliva samples were collected at 19 months (mean [SD], 18.85 [0.74] months) for 466 twins before testing, 474 twins after testing, and 423 children at both times. Interviews regarding environmental variables were conducted with the mother in 99.7% of cases. The competitive situation was done on a subset of this sample. Three hundred and forty six participants completed the first saliva sample and the competitive situation, and three hundred and twenty two participants completed both saliva samples (before and after the exposure to unfamiliar situations) and the competitive situation.

Procedures and measures

Saliva collection

Salivary cortisol sampling is a non-invasive and valid way to assess HPA axis activity (Kirschbaum and Hellhammer, 1989; Schwartz et al., 1998). All saliva samples were obtained between 8:05 AM and 12:15 PM during the 19 months old laboratory visits. Mothers were instructed not to give their child anything to eat or drink 20 minutes before each sampling time. As illustrated in Fig. 1, saliva was collected (Salivette; Sarstedt, Nümbrecht, Germany) prior to and following the participation to two unfamiliar situations known to be moderately stressful at that age (Goldberg et al., 2003; Mullen et al., 1993; van Bakel and Riksen-Wairaven, 2004). In the first situation, one twin and the mother were alone in a corner of a room when a woman dressed as a clown entered the room, went to the opposite corner, and invited the child to approach by offering a set of familiar toys. In the second situation, a noisy, odd-looking, moving toy robot was placed on a platform in the opposite corner of the room. Each session lasted 280 seconds separated by 5 minutes of mother–child free play. As shown in Fig. 1, the pre-test sample was obtained at the arrival at the lab. The post-test sample was obtained 20 minutes after the end of the procedure in order to capture the peak cortisol response (Ramsay and Lewis, 2003). Saliva samples were stored at −20 °C until analysis. All samples were analyzed in a single batch using radioimmunoassay (Diagnostic Systems Laboratories Inc, Webster, Texas). Intra-assay variability was less than 10%.

The competitive situation: pair encounters with an unfamiliar peer

The competitive situation took place 30 minutes (31.2, s.d. 26.4) after the end of the exposure to unfamiliar situations. The aim of the procedure was to assess the toddlers' reactions when confronted to a potentially competitive situation with an unfamiliar peer. The entire session was videotaped. We organized the subjects into 197 pairs of unfamiliar twins, irrespective of zygosity and gender. Each co-twin from one set (A1, B1) was paired with one co-twin from another set (A2, B2), yielding 2 unfamiliar pairs: (A1, A2) and (B1, B2).

An experimenter invited the mothers and a first twin to come into a 5 × 5-meter room equipped with a video-camera. The experimenter explained that the aim of the task was to see how children play with each other, and to see whether they would share toys. The experimenter then asked the mothers to make their child sit at a precise place marked on the floor, and to sit behind the child. A 50-centimeter distance separated the children from one another.

The situation comprised four sessions. Each session lasted 30 seconds and took place in the following way: mothers were asked to hold their child. Then, the experimenter took out an attractive toy from her pocket and drew the attention of the children toward the toy. The toy was either a red spinning top, a little blue and red car, a yellow radio, a phone, or a red rattle. The experimenter then placed the toy on the ground at an equal distance from the two

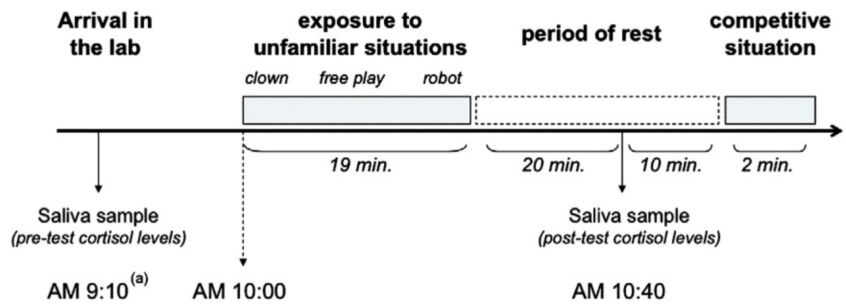

Fig. 1. Schematization of the experimental procedure used to assess cortisol levels in children exposed to the unfamiliar and dominance situation. Note: average time.

children. The mothers were told to let their child move and not to interact with him or her after the toy was placed on the ground. After 30 seconds, the experimenter retrieved the toy either from the child who had taken it or from the floor. Finally, the mothers were asked to sit their child on the mark, and the session was repeated three times, each time with a new toy. Thus, each twin participated to four successive competitive sessions with the same unfamiliar twin. The toys were equally appealing to the boys and to the girls.

We measured of social dominance based on the method developed by Charlesworth and La Freniere (1983) and adapted to dyads (Plusquellec et al., 2007). For each individual, we quantified the proportion of time the child accessed the resource, in this case the toy (got the toy). In order to determine whether any significant relation between cortisol levels and the ability to control the resource might be attributable to emotional distress in the competitive situation, we quantified the frequency of crying as a proxy of emotional distress.

In order to establish inter-coder reliability and to avoid inter-observer contamination (e.g., risk of attributing the same behavior to identical twins), the interactions were videotaped. One person coded the interactions of all the B twins (B1, B2, B3…B197) and 42 A twins, and the other one coded those for all of the A twins and 42 B twins. Then, we used those coded by both observers to calculate inter-coder reliability. Kappa coefficients between the coders' ratings were 0.85 for “get the toy” and 0.95 for “crying.” Social dominance for a child was defined as the proportion of time the child controlled the resource (“got the toy”).

Data analyses

The present study aims to describe the association between two observed characteristics rather than the genetic and environmental etiology of those characteristics. However, because twin pairs share common genetic factors and are growing up in the same families, they tend to be more phenotypically alike than unrelated individuals selected randomly from the general population. We therefore controlled for the non-independence of the observations using the Huber–White robust sandwich estimator for standard errors (Huber, 1967; White, 1982). The Huber–White robust sandwich estimator is implemented in the procedure Proc Genmod (SAS 9.1, SAS Institute Inc.) using the method of generalized estimating equations (GEE, (Liang and Zeger, 1986)). Because the GEE analysis is only valid if the assumption of missing completely at random (MCAR) is met (Little and Rubin, 1989), we tested it using the Little's test (Scheffer, 2002) implemented in SPSS version 13 (Statistical Package for Social Sciences, SPSS Inc). Data were imputed using the full information maximum likelihood (FIML) with the Mplus statistical package (Version 6, Muthén and Muthén, 2010) that provides FIML estimator and the standard Huber–White robust procedure that takes into account the dependence of twin pair data.

The cortisol pre-test level, post-test level and the reactivity ratio score were examined for outliers, defined as values ± 3 standard deviations from the mean (Grunau et al., 2004; Gunnar et al., 1989; Ramsay and Lewis, 2003). The analyses were run on winsorized distributions. Percentage of missing data for all variables ranged from 3.1% to 22%. Little's test showed that the missing values for pre-test cortisol level were not missing completely at random (MCAR) ($\chi 2$ (27, $N=311$) = 43.52, $P<.05$), but were probably missing at random (MAR), suggesting that what caused the data to be missing does not depend upon the missing data itself. We thus decided to use Mplus (Version 6, Muthén and Muthén, 2010) MLR estimator to adjust data dependence and missing pattern together.

Results

Preliminary analyses

The mean proportion of time the child controlled the resource (“got the toy”) was 34.3% (s.d. 29.7). The frequency of crying during the competitive situation ranged from 0 (80.7% of participants) to 7 (mean [SD]: 0.34 [0.91]). Preliminary analyses performed on frequency of crying in response to the task revealed that there was no sex differences in the frequency of crying in the competitive situation ($Z=0.56$, $P=0.57$), and being paired with a girl or a boy did not influence the frequency of crying in the competitive situation ($Z=-0.95$, $P=0.34$). As well, no association was observed between the frequency of crying and the ability to get the resource in the competitive situation ($r_{388}=-0.05$; $P=0.34$).

The proportion of time the child got the toy during the competitive situation was not associated with the interval of time between the end of the unfamiliar situation and the start of the competitive situation (respectively, $r_{372}=0.01$; $P=0.84$; $r_{363}=-0.08$; $P=0.11$). As well, there were no sex differences for the proportion of time the child “got the toy” ($Z=-1.23$, $P=0.22$), and being paired with a girl or a boy did not influence the proportion of time the child “got the toy” ($Z=0.20$; $P=0.84$). The interval between the unfamiliar situation and the competitive task did not differ between sexes ($Z=0.76$; $P=0.45$).

Cortisol levels

When testing cortisol reactivity to the unfamiliar situation in the entire group of children, no significant cortisol increase was found between the pre-test (mean [SD]: .39 μg/dL [.27]) and the post-test levels (.37 [.22]) ($Z=-1.57$, $P=.12$). A reactive cortisol ratio was computed according to the law of initial values (Lacey, 1956), which takes into account the dependency of the post-test values on the initial values. According to the law of initial values, the change score should be adjusted if the correlation between the initial value ($t1$) and the change score ($t2-t1$) is negative, which was the case ($r_{322}=-0.69$; $P<.001$) (Lacey, 1956; Lewis and Ramsay, 1999; Ramsay and Lewis, 2003). A cortisol reactivity ratio was thus computed by dividing ($t2-t1$) by $t1$ (Luby et al., 2003). The pre-test cortisol levels was negatively related to the reactive cortisol ratio ($r_{322}=-0.50$, $P<0.00$), indicating that the higher pre-test cortisol levels a child had, the lower reactivity he/she showed following the unfamiliar situation.

Over the entire group of children, neither pre-test cortisol levels nor reactive cortisol levels were associated with the frequency of crying ($r_{336} = -0.02$, $P = 0.7$ and $r_{312} = 0.08$, $P = 0.14$, respectively), suggesting that any association between cortisol levels and resource control could not be explained by differences in emotional distress.

Pretest cortisol and resource control

The time of day when saliva was collected was not associated with pre-test cortisol levels ($r_{344} = -0.04$, $P = 0.44$) and no sex differences were observed on pre-test cortisol levels ($Z = -0.69$, $P = 0.49$). Over the entire group of children, pre-test cortisol levels were not associated with the proportion of time the child got the toy ($r_{346} = -0.04$, $P = 0.45$). However, when assessing this relationship in boys and girls separately, we found that in boys increased pre-test cortisol levels were significantly associated with decreased proportion of time the boys controlled the resource/got the toy ($r_{174} = -0.17$, $P = 0.02$, Fig. 2). This association was not found in girls ($r_{172} = 0.09$, $P = 0.21$).

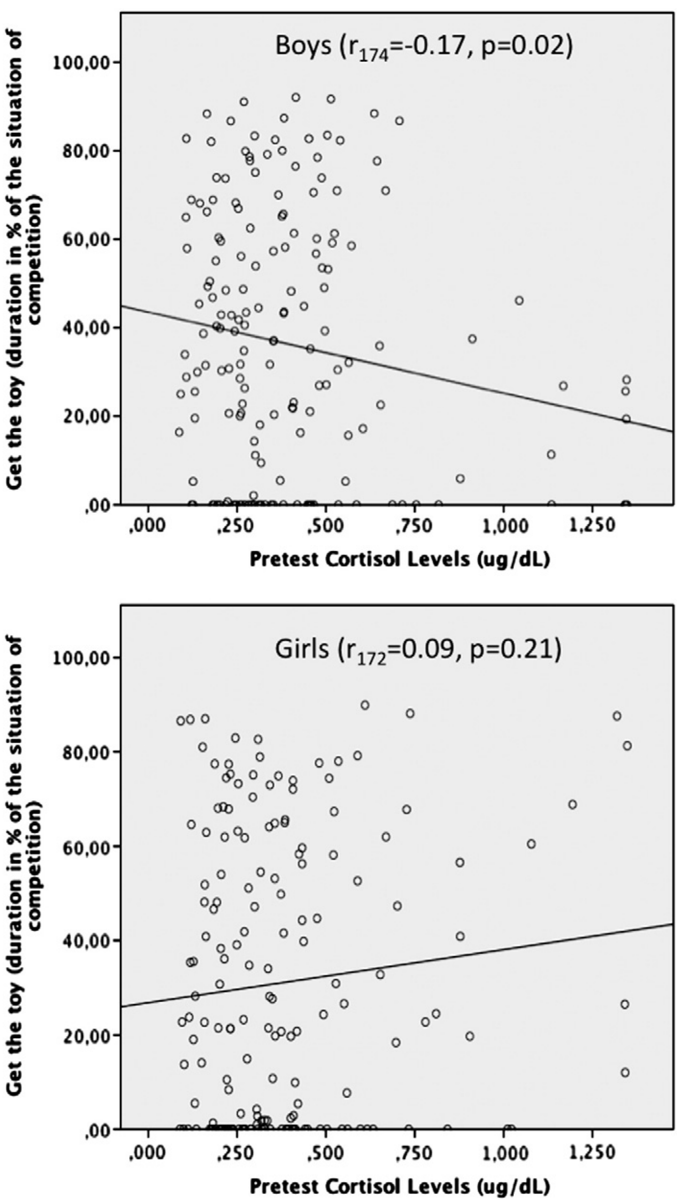

Fig. 2. Association between pre-test cortisol levels and the proportion of time the child "got the toy" in the competitive situation according to sex.

Reactive cortisol ratio and resource control

The time of day when saliva was collected was not associated with the reactive cortisol ratio ($r_{320} = -0.02$, $P = 0.72$) and no gender differences were observed on the ratio ($Z = 0.63$, $P = 0.53$). Over the entire group of children, no significant associations were found between the reactive cortisol ratio and the proportion of time the child got the toy ($r_{322} = -0.04$, $P = 0.51$). No significant associations were found between reactive cortisol ratio and behavioral outcomes even when analysis was split by sexes.

Discussion

In the present study, the hypothesis that lower glucocorticoids levels are associated with higher resource control in a competitive situation between two unfamiliar peers that have limited social experience was tested in boys and girls. In 19-month-old boys, high glucocorticoids levels before the unfamiliar situations (pre-test cortisol levels) were significantly related to a low proportion of time the boy controlled the resource in the competitive situation, which is in agreement with our hypothesis. In contrast, we did not find any association among girls. No association was detected between the frequency of crying and the capacity to control resource in the competitive situation for both girls and boys, suggesting that the association observed between resource control and cortisol in boys was not accounted for by sex differences in emotional distress.

Sex differences in cortisol reactivity have already been documented in young adulthood (Kirschbaum et al., 1992) or in elderly subjects (Kudielka et al., 1998) exposed to a social stressor, but no apparent sex differences in children, a fortiori in toddlerhood, have been yet reported (Buske-Kirschbaum et al., 2003; Khilnani et al., 1993; Lundberg, 1983; Tout et al., 1998), which is in agreement with our results. As to emotional and social behaviors, several studies have looked at sex differences across childhood. Results showed the absence of sex differences in rates of temperamental problems during the first few years of life (Earls and Jung, 1987; Maziade et al., 1984; Prior et al., 1993). No sex differences in rates of behavioral inhibition to an unfamiliar situation were neither reported in toddlerhood (Kagan et al., 1989), and differences between sex on the Child Behavioral Checklist, used to assess behavioral and emotional problems, reached statistical significance only from 4 years of age (Achenbach et al., 1987). The absence of sex differences in our settings for 19-month-old children is thus in agreement with this body of knowledge.

Pre-test cortisol levels have been considered for a long time as reflecting basal cortisol levels, but new studies in humans of various ages now suggest that the environmental context of testing can induce a stress response in many individuals, and have a significant impact on the pre-test cortisol values (Balodis et al., 2010) and the cortisol reactivity ratio measured after exposure to a laboratory stressor (for a review, see Lupien et al., 2007). In our case, we observed a significant negative correlation between pre-test cortisol levels and reactive cortisol ratio which could be explained by an activation of the hypothalamic–pituitary–adrenal axis prior to the unfamiliar situation. This activation would result in an increased pre-test cortisol levels caused by the stressful characteristics of the lab setting for the 19 month-old participants, such as novelty or unpredictability. Very few studies have looked at cortisol reactivity in toddlerhood and the unfamiliar situation may fail to elicit stress above and beyond that which may have inadvertently been elicited by the events preceding the unfamiliar situation, that is the arrival in the lab. Indeed, we know that at this point of development, individual differences have already emerged in fearful inhibition to novel or intense stimuli (Kagan et al., 1988), and that distress to novelty is associated with increased cortisol in toddlerhood (Blair et al., 2008). In this case, the association found in boys between pre-test cortisol levels and resource control in the competitive situation could rather reflect stress effects on resource control.

If indeed the pre-test cortisol levels obtained in this study are reflective of a cortisol stress response to the novelty of the laboratory environment, then the significant association between pre-test cortisol and resource control observed in boys would suggest that boys may recover from the physiological reactivity induced by the lab setting more slowly than girls. Fig. 2 shows that at low-to-moderate pre-test cortisol levels, girls and boys seemed to be equally able to control the resource; however, at high pre-test cortisol levels, boys exhibited more difficulties to control the resource while girls appeared unaffected. Although pre-test cortisol levels and reactive ratio did not differ between boys and girls, we speculate that a slower recovery in boys may explain the apparently unaffected resource control in girls at high pre-test cortisol levels in comparison to boys. Indeed, the HPA axis activation could take more time in boys to recover than in girls, and this could impact on the capacity of boys to control resources when tested in the resource control situation. This hypothesis of delayed recovery from stress in boys may explain the lack of sex differences in both pre-test and reactive cortisol levels, although pre-test levels of cortisol were found to be associated with resource control in boys while this was not the case in girls. However, because few children had high pre-test cortisol levels, this finding need to be replicated in a larger group of boys and girls with higher cortisol levels or in the context of a more stressful laboratory paradigm.

Considering that the pre-test cortisol levels are more of a proxy of the HPA axis activation following the arrival in the lab, our result indicates that boys with lower HPA reactivity would have more chance to be resource controller than boys with higher HPA reactivity. In the context of Sapolsky's (Sapolsky, 2005) predictions for stress responses to hierarchical position in stable and unstable social systems, our result was unexpected since this relation is usually observed in stable hierarchies, which is unexpected in children with limited social experience and a fortiori in our setting. For example, in the West et al.'s (2010) study, only the scholastic hierarchy was significantly inversely related with cortisol, cortisol increasing with lower position, while, more unstable hierarchy, such as sport hierarchy was associated with cortisol in the opposite way, elevated cortisol levels being associated with bottom position. In the Hellhammer et al. (1997) study, done with army recruits during the boot camp training period (unstable hierarchy), higher increased in salivary cortisol levels following psychological stress, was observed in socially dominant subjects in comparison with subordinate ones. Our result may be explained by two complementary points: firstly, children as young as 19 months of age may have experienced social interactions (in the daycare, or in the family) that have increased their social skills, and thus would have reduced their HPA reactivity; and secondly, high pre-test cortisol levels may reveal high physiological reactivity that have been associated with certain behavioral traits such as behavioral inhibition (Byrne and Suomi, 2002; Kagan et al., 1988), and thus a lower ability to control resource.

The present study has limitations that must be acknowledged. First, the cortisol reactivity was based on single pre-test and post-test saliva samples. Further studies may benefit from collecting additional saliva samples at home to ascertain more precisely the basal cortisol levels at home. Also the collection of more saliva samples following the stressful situation could have allowed the assessment of the ability of the HPA system to recover. Second, for ethical reasons, a rather mild yet developmentally relevant stressful situation was used (Buss et al., 2004), resulting in an absence of increase in cortisol overall. Many factors may have accounted for this general lack of increase in cortisol, including an already high level of cortisol before testing because of the laboratory context. Lower cortisol reactivity may also be due to the timing of the post-test saliva sample collection that could have been done earlier. Indeed, cortisol usually peaks 20 minutes after the onset of a psychological stressor (Kirschbaum et al., 1999; Kirschbaum et al., 1993), which suggest that we could have missed this peak. Third, the competitive situation is very brief and although it has been previously validated (Plusquellec et al., 2007), it represents only part of the toddler's social functioning, that is the ability to control resource in a particular context. Studies have shown that other important factors account for social functioning in toddlers, such as their ability to use different behavioral strategies to access resources as a function of the context (verbal or physical behaviors, (Charlesworth and Dzur, 1987); cooperation, (Green and Rechis, 2006; Hawley, 1999)).

In conclusion, the present study is the first to reveal a significant association between cortisol levels and the ability to control resource in 19 month-old boys. The ability to regulate their emotional reactivity appears as a significant factor in boys to control resource in the context of a swift encounter with an unfamiliar peer. On the other hand, in girls, this ability to regulate emotional reactivity does not seem to impair their success in the same situation. Next step could be to take advantage of the genetic particularity of this sample in order to explore whether the association observed between lower resource control and higher pre-test glucocorticoids secretion is environmentally-mediated (laboratory context, familial environment) or could be explained by genetic factors.

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