

**RELATION OF DAILY PATTERNS IN SALIVARY CORTISOL TO PEER AND  
TEACHER RELATIONSHIPS AND SOCIAL BEHAVIOURS IN MIDDLE  
CHILDHOOD**

by

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## ABSTRACT

In the published literature, the association between salivary cortisol and aggressive behaviours in children is equivocal. This has provoked questions about the potential role that other factors, such as supportive relationships with peers and teachers, may play in mediating the association between cortisol and behaviour. This study was designed to investigate the association between various indicators of daily patterns in cortisol (i.e., diurnal slope, average morning, noon, and afternoon cortisol) and aggressive and prosocial (sharing and helping) behaviours in a non-clinical cohort of school-aged children in an everyday classroom context. It was hypothesized that lower cortisol would be significantly associated with higher levels of proactive, reactive, and socially aggressive behaviours and that this association would be uniquely mediated by peer acceptance and teacher closeness. This study also explored the association between cortisol and prosocial behaviours. Salivary cortisol was obtained from children ( $N = 89$ , *Mean* age = 10.4 years, Range, 9.2 – 12.2 years) in a classroom setting three times a day (9am, 12pm, and 3pm) across four consecutive days. Multiple informants (i.e., peers and teachers) completed questionnaires on children's social behaviour, peer acceptance (peers only), and student-teacher closeness (teachers only). Social behaviours were individually regressed on various indicators of daily patterns of cortisol, controlling for age and gender. Findings revealed inverse relations of afternoon (3pm) cortisol to reactive, proactive, and social aggression. Positive relations of afternoon cortisol to prosocial behaviour, peer acceptance, and teacher closeness were found. A series of independent multiple mediation analyses demonstrated a unique mediating influence of peer acceptance and, separately, teacher closeness. Peer acceptance and teacher closeness uniquely mediated the association between afternoon cortisol and teacher- and peer-reported prosocial behaviours, and teacher-reported proactive aggression. In addition, lower peer

acceptance mediated the association between low afternoon cortisol and higher teacher-rated reactive and social aggression. The findings from this research contribute to the growing body of knowledge on associations among children's daily cortisol patterns, social behaviours, and peer and teacher supportive relationships in a classroom context. These results suggest that an important direction for future research is the incorporation of neurobiological measures of behavioural development into classroom-based research.

## **PREFACE**

Collaborators are the following:

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## **CHAPTER 1**

### **Introduction**

The past few decades have seen a plethora of theoretical and empirical research aimed at elucidating the mechanisms by which positive social, emotional, and behavioural adaptation can be promoted during childhood. Nonetheless, approximately 20% of Canadian youth continue to suffer from mental health problems with rates predicted to increase up to 50% by the year 2020 (Canadian Paediatric Society, 2006). The significant cost to the individual and family, combined with an economic burden estimated at a staggering \$30 billion, places mental health and behavioural problems among the most costly conditions in Canada (Canadian Paediatric Society, 2006; Stephens & Joubert, 2001).

Middle childhood, the ages between 6 and 12, represents a time of increased anxiety and stress, and the emergence or consolidation of aggressive behavioural problems (Aber, Brown, & Jones, 2003; Kowaleski-Jones & Duncan, 1999; Loeber & Hay, 1997). Whereas some degree of aggression is age-normative, variations in normal development across childhood may eventually produce highly aggressive individuals (Côté, Zoccolillo, Tremblay, Nagin, & Vitaro, 2001; Loeber, 1997). Research shows that individuals displaying high levels of aggression in middle childhood are significantly more at risk for long-term behavioural problems and maladaptation (Brame, Nagin, & Tremblay, 2001; Lerner, Hertzog, Hooker, & Hassibi, 1988; Loeber, 1991; Reiss & Roth, 1993).

Research has identified a number of factors significantly associated with childhood aggressive behaviours including, internal neurobiological processes, such as activity of the hypothalamic-pituitary-adrenal (HPA) axis, and external contextual factors, such as supportive relationships with others. Of the research examining the neurobiological underpinnings of aggression in children, the majority of studies have indexed HPA axis activity using salivary measures of the hormone cortisol (e.g., Alink, van IJzendoorn, Bakermans-Kranenburg,

Mesman, & Juffer et al., 2008; Bora, Yucel, & Allen, 2009; van Goozen & Fairchild, 2006). Cortisol exhibits a typical diurnal pattern of secretion, with levels rising within 20 - 45 minutes after waking, then gradually declining across the day. Variations in children's daily patterns of salivary cortisol<sup>1</sup>, such as slow decreases across the day or increases later in the day, may be indicators of short- or long-term dysfunction (see Gunnar & Vasquez, 2001; Jessop & Turner-Cobb, 2008). The burgeoning number of investigations examining HPA axis activity and aggressive behaviours in children in recent years is likely due to the ease of sampling cortisol in children's saliva (for recent reviews see Alink et al., 2008; Gunnar & Quevedo, 2007; Jessop & Turner-Cobb, 2008). However, the existing research examining the association between daily patterns of salivary cortisol and aggression in middle childhood is limited in three important ways. First, most investigations in this area have used a variety of different indicators of daily patterns in salivary cortisol (e.g., average values at different times of the day, diurnal slope or change across the day) obtained in diverse settings (e.g., home, laboratory, day camp) under different conditions (e.g., resting or in response to an experimentally-induced stressful task). Limited research has measured multiple samples of cortisol in typically developing children taken at rest throughout the day in an everyday classroom context (see however Lupien, King, Meaney, & McEwen, 2001). Second, minimal research has examined daily patterns of cortisol in relation to specific subtypes of aggressive behaviours, such as proactive, reactive, or social aggression; behaviours that are shown to be differentially related to children's adaptation (see Little, Henrich, Jones, & Hawley, 2003). Moreover, we could find no research examining the association between cortisol and prosocial (sharing, helping) behaviours in middle childhood. Finally, many studies have failed to examine the potential role that supportive relationships with

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<sup>1</sup> The term "daily patterns in salivary cortisol" is used throughout this review to represent indicators of salivary cortisol obtained under resting conditions in a non-experimentally stimulated setting.

peers and teachers<sup>2</sup> may play in mediating the association between daily patterns of salivary cortisol and social behaviours. Research indicates that by middle childhood, children's HPA axis functioning, represented by daily patterns in cortisol, may influence their ability to form positive supportive relationships with peers (e.g., Gunnar, Sebanc, Tout, Donzella, & van Dulmen, 2003; Vaillancourt et al., 2008). In turn, being accepted by peers and having close, supportive relationships with teachers is shown to have an immediate and enduring impact on children's behavioural adaptation (Juvonen & Wentzel, 1996; Wentzel, Barry, & Caldwell, 2004; Wentzel, 2005; Wentzel & Erdley, 1993). However, the ways in which children's daily patterns in cortisol are associated with peer and teacher relationships and subsequent behaviour have yet to be explored.

Recent multidisciplinary theories of human development advocate the use of complex analytical models that address the interplay of variables in distinct levels of biological, behavioural, and relational-contextual organization (see Gunnar & Quevedo, 2007; Lerner, 2006; Masten et al., 2004; Sameroff, 1987, 2009; Susman & Ponirakis, 1997). Accordingly, building on an integrative theory of childhood social behaviour, this research was designed to examine the associations of daily patterns in salivary cortisol to peer- and teacher-rated reactive, proactive, and social aggression, and prosocial behaviours, and to investigate the way in which children's supportive relationships with peers and teachers mediates the association between daily patterns of salivary cortisol and social behaviour during middle childhood. Understanding how daily HPA axis functioning is associated with children's social relationships and behaviours during middle childhood will not only contribute to the extant empirical research in the field, but will provide important direction for the incorporation of neurobiological measures of behavioural development into classroom-based research.

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<sup>2</sup> Supportive relationships with peers and teachers were defined as: peer acceptance (e.g., "How much do you want to be in activities with this classmate?"), and teacher-reported student-teacher closeness (e.g., "I share a warm and affectionate relationship with this child").

## **The Developmental Significance of Middle Childhood**

Middle childhood is a developmentally important stage in children's lives characterized by a rapid increase in cognitive and social development (Collins, 1984; Sameroff & Haith, 1996), and an increasing focus on peers and social acceptance (Brown, 1990; Wigfield, Byrnes, & Eccles, 2006). The middle childhood years are critical for acquiring the knowledge and social skills necessary for negotiating the more socially challenging nature of adolescence and young adulthood (Flinn, 2006; Geary, Byrd-Craven, Hoard, Vigil, & Numtee, 2003). Collins (1984) posits that it is during the middle childhood years that children's personalities, behaviours, and competencies may consolidate into forms that persist into adolescence and adulthood. Similarly, research indicates that although there is variation among children in terms of rate of growth and development during this period, middle childhood development is a powerful predictor of adolescent adjustment and academic success (Huston, & Ripke, 2006). Deviations from normative processes during this critical stage of development are shown to have a dramatic impact on long-term adaptation (Sroufe, Egeland, Carlson, & Collins, 2005; Trentacosta & Shaw, 2009). In particular, the emergence or consolidation of aggressive behaviours during middle childhood has a deleterious impact on children's current and future socio-emotional, academic, and physical adaptation. For example, numerous studies demonstrate that aggression in middle childhood is related to poor school achievement (Brook & Newcomb, 1995; Malecki & Elliot, 2002; Wentzel, 1993), peer rejection (Coie, Lochman, Terry, & Hyman, 1992), antisocial behaviour (Coie & Dodge, 1998), long-term physical violence, criminal behaviour, and unemployment (Cairns & Cairns, 1994; Fergusson & Horwood, 1998; Kokko & Pulkkinen, 2000; Kokko, Tremblay, Lacourse, Nagin, & Vitaro, 2006; Loeber & Hay, 1997; Rutter, Giller, & Hagell, 1998). An important assumption of these predictive models is that aggressive behaviours and their underlying risk factors are stable during the course of development. Indeed,

empirical research supports the claim that aggressive behaviours are relatively stable from childhood to adolescence and beyond (Vitaro et al., 2006).

Generally the development of aggressive behaviours has received more attention than prosocial behaviours in the developmental literature, likely due to the significant social costs to the individual associated with this behaviour (Coie & Dodge, 1998; Kokko et al., 2006). However, sharing, helping, and cooperative behaviours are also predictive of unique developmental trajectories during middle childhood. These prosocial behaviours are associated with emotional competence, positive relations with peers and teachers, and increased academic achievement (e.g., Caprara, Barbaranelli, & Pastorelli, 2001; Eisenberg, Fabes, Karbon, & Murphy, 1996; Eisenberg et al., 2002; Eisenberg et al., 1999; Hymel, Schonert-Reichl, & Miller, 2006). Hence, research aimed at better understanding the external contextual and internal biological correlates of *both* prosocial and aggressive behaviours during middle childhood has vital implications for promoting children's long-term adaptation (Malecki & Elliot, 2002). Understanding the mechanisms that underlie changes in specific subtypes of aggressive behaviour may help tailor effective interventions to ameliorate aggressive behaviour and improve mental health outcomes across middle childhood. A critical first step in this research process is to distinguish among the distinct forms and functions of children's social behaviours.

### **Social Behaviours in Middle Childhood**

The traditional definition of aggression is behaviour intended to hurt, harm, or injure another person (Coie & Dodge, 1998). More recently however, aggressive behaviours in children and adolescents have undergone an important conceptual and definitional modification in the field (Vitaro, Brendgen, & Barker, 2006). Research has shown that children may engage in a variety of subtypes of aggressive behaviours that separate into different functions (e.g., proactive and reactive aggression) and forms (e.g., physical and social aggression) of aggressive behaviour that are differentially related to adaptation (Little et al., 2003; Murray-Close & Ostrov, 2009;

Vitaro et al., 2006). As such, research designed to examine the developmental trajectories, antecedents and consequences of aggressive behaviour need to distinguish among subtypes of aggressive behaviour (Vitaro et al., 2006).

**Proactive and reactive aggression.** Reactive aggression is defined as defensive and retaliatory aggression, characterized by a highly aroused aggressive response to a real or perceived provocation (Dodge, 1991; Kempes, Matthys, de Vries, & van Engeland, 2005). Proactive aggression, in contrast, is defined as planned, goal-directed, low-arousal behaviour focused on an anticipated goal such as material possession or social dominance (Dodge, 1991; Dodge & Coie, 1987). Debate exists in the literature as to whether these two subtypes represent distinct or related dimensions of aggression (see Hubbard, McAuliffe, Morrow & Romano, 2010 for a recent review). Originally, researchers hypothesized that two distinct subgroups of aggressive children existed; one group displaying primarily proactive aggression and the other group reactive aggression (see Dodge, 1991). According to a recent overview by Hubbard and colleagues (2010), evidence suggests that proactive and reactive aggression tend to co-occur, with most aggressive children displaying some degree of both subtypes of aggression. To illustrate, high correlation exists between measures of proactive and reactive aggression across samples of youth, ranging from .40 to .90, with a typical estimate of .69 (see Card & Little, 2006 for a meta-analysis). Hence, Hubbard and colleagues (2010) propose that these subtypes of aggression are more accurately depicted as continuous dimensions that exist within a child, rather than as discrete categories. Despite this characterization of proactive and reactive aggression as part of a continuum, the distinction between the two subtypes is still useful.

Proactive and reactive forms of aggressive behaviour have distinct theoretical frameworks or social information patterns (Crick & Dodge, 1994, 1996), originate in different familial and social contexts, and are differentially related to specific indices of psychosocial adjustment in childhood and adolescence (Card & Little, 2006; Card, Stucky, Sawalani, & Little,

2008; Hubbard et al., 2010; Polman, de Castro, Koops, van Boxtel, & Merk, 2007). A series of longitudinal investigations of familial precursors to adolescent outcomes of proactive and reactive aggression in Canadian youth have demonstrated that proactive and reactive aggression predict different forms of aggressive and antisocial behaviours. For example, early proactive aggression is shown to predict later delinquency, while early reactive aggression predicts later dating violence (Brendgen, Vitaro, Tremblay & Lavoie, 2001; see Hubbard et al., 2010; Raine et al., 2006; Vitaro, Brendgen & Tremblay, 2002). Furthermore, different familial contexts are shown to influence the emergence of proactive and reactive aggression as children develop. Specifically, parental substance abuse and lack of parental supervision are shown to influence the association between early proactive aggression and delinquency, whereas physical abuse and lack of maternal caregiving influence the association between early reactive aggression and later dating violence (Brendgen et al., 2001). Taken together, these findings demonstrate the importance of social contexts and supportive relationships in differentially predicting proactive and reactive subtypes of aggression in childhood. What is lacking in the literature is an investigation into the role of non-familial relationships, such as those with peers and teachers, in the emergence or consolidation of proactive and reactive aggression. Evidence suggests that children displaying proactive aggression may be rejected by their peers for different reasons than children displaying reactive aggression. For example, children rated as reactively aggressive are described as 'hot headed' and tend to be rejected by their peers (Dodge & Coie, 1987; Vitaro et al., 2002), and report more depressive symptoms and social anxiety than proactively aggressive children (Day, Bream, & Pal, 1992; Morrow, Hubbard, McAuliffe, Rubin, & Dearing, 2006; Raine et al., 2006). With respect to gender differences, the majority of investigations into physical forms of aggression have been conducted primarily on boys (Card & Little, 2006). Of the relatively few studies with girls, there is evidence that both proactive and reactive aggression

can be problematic for girls across middle childhood (Crapanzano, Frick, & Terranova, 2010; Marsee & Frick, 2007).

Emerging evidence suggests that proactive and reactive aggression may not only stem from different social information processing models, but from different neurobiological functioning as well. Theory suggests that reactive aggression is characterized by high levels of frustration, anger and acute activity of the HPA axis (Berkowitz, 1989), whereas proactive aggression is thought to be associated with stimulation-seeking and biological under-arousal or low HPA axis activity (e.g., Hubbard et al. 2002). However, as will be discussed in a later section, preliminary investigations into the neurobiological correlates of proactive and reactive sub-types of aggression in children are limited by a number of methodological issues (e.g., Murray-Close et al., 2008). The most salient concern is the lack of examination of children's HPA axis activity (i.e., daily patterns of salivary cortisol) in everyday classroom contexts. In addition, limited published studies exist examining the influence of peer and teacher supportive relationships on the association between salivary cortisol and proactive and reactive aggression.

**Social aggression.** Social aggression is a significant and ubiquitous form of childhood aggression that has only in recent years received the attention it deserves. An increase in empirical research combined with a surge in popular press has sparked interest in socially aggressive behaviours such as gossiping, rumour-spreading, and social exclusion that are intended to harm others through damaging social relationships (Cairns, Cairns, Neckerman, Ferguson, & Gariépy, 1989; Galen & Underwood, 1997). Social aggression during middle childhood is defined as behaviours aimed at damaging another child's self esteem or social status (Underwood, 2003). Also described as relational aggression (Crick & Grotpeter, 1995), or indirect aggression (Feshbach, 1969; Lagerspetz, Björkqvist, & Peltonen, 1988), these forms of aggression are essentially the same (see Neal, 2010). However, some researchers continue to make the distinction between relational, social, and indirect aggression based on slightly

different aspects of, or strategies by which, the harm is delivered (see Ostrov & Godleski, 2010; see also Underwood, Galen, & Paquette, 2001 for a debate on shared and unique features).

Social aggression is the preferred term as it encompasses a broader range of subtle aggression that includes non-confrontational (i.e., gossiping) and confrontational (i.e., threats to end a friendship) behaviours (see Neal, 2010).

Debate exists in the literature as to whether girls rate higher on social aggression than boys. Some researchers claim that girls are as aggressive as boys during middle childhood, with boys displaying more physical aggression and girls displaying more social aggression (Bjorkqvist, Lagerspetz, & Kaukiainen, 1992; Crick et al., 1999). However, findings from longitudinal research are contradictory, showing; no gender differences in measures of teacher reported social aggression in children followed from age 9 to 13 years (Underwood, Beron, & Rosen, 2009), increased social aggression across one calendar year for girls only (Murray-Close, Ostrov & Crick, 2007), or even that boys are more socially aggressive than girls (e.g., Leadbeater, Boone, Sangster, & Mathieson, 2006; Salmivalli & Kaukiainen, 2004). Indeed, a recent meta-analysis by Card and colleagues (2008) suggests that the small gender difference observed favouring girls, in terms of prevalence of social aggression, was in fact trivial. What appears to be influential in predicting social aggression in middle childhood is children's maturation in socio-cognitive abilities (i.e., language skills, memory) and influence of social context such as friendships and peer interactions.

Evidence shows that social aggression emerges in the preschool years, peaks around middle childhood (Crick, Casas, & Mosher, 1997; Murray-Close et al., 2007; Vaillancourt, Miller, Fagbemi, Côté, & Tremblay, 2007) and is moderately stable across early adolescence (Godleski & Ostrov, 2010). The increase in social aggression during middle childhood is likely due to the increased importance that peer relationships play during this developmental period (Bjorkqvist et al., 1992; Buhrmester, 1996; Underwood, Beron & Rosen, 2009). Researchers have

found that children tend to use socially aggressive behaviours within the context of close, intimate friendships. Sharing of intimate, personal information between friends provides increased opportunity for socially aggressive behaviours that manipulate another through relationship-damaging tactics such as gossip and rumour-spreading. As such, as children spend more time with peers across middle childhood and close friendships emerge, social aggression correspondingly increases. To illustrate, research shows an increase in social aggression in elementary school girls across one calendar year that is associated with increases in close friendships (Murray-Close et al., 2007). In addition, children in peer groups highly supportive of social aggression are shown to become increasingly aggressive (see Werner & Hill, 2010). For example, longitudinal research in 8 to 13-year-olds shows that the transition to middle school is marked by increased approval of social aggression by peers. Theory suggests that as children acquire more socio-cognitive skills and spend more time with peers and close friends, they display more sophisticated and frequent displays of social aggression (Crick et al., 1999). Increased cognitive capacities may permit children to recall specific relationship history and to retaliate in response to past behaviours (Murray-Close et al., 2007). It is also suggested that as children mature they become less tolerant of physically aggressive behaviours and employ subtler forms of social aggression as tools to secure social standing during middle childhood. This is especially true of socially aggressive behaviours such as social exclusion and rumour-spreading that require sophisticated coordination of social networks (see Neal, 2010 for an overview of social correlates of social aggression). As Rose and colleagues state, “The ability to aggress strategically in ways that are socially dominant, that display superiority, and that result in perceived popularity likely requires advanced interpersonal skills that may develop with age” (Rose, Swenson & Waller, 2004, p. 385).

Several cross-sectional and longitudinal studies that have compared the relationship of social aggression to social status and peer nominated popularity in children across middle

childhood have generally found that while socially aggressive children are often perceived as higher in social status or popularity, they are not well liked by peers (Cillessen & Mayeux, 2004; Crick & Grotpeter, 1995; LaFontana & Cillessen, 2002; Prinstein & Cillessen, 2003; Vaillancourt & Hymel, 2006; Neal, 2010, p. 127). Furthermore, social aggression is linked to poor adjustment across the elementary years (Crick, Ostrov, & Werner, 2006; Heilbron & Prinstein, 2008; Murray-Close et al., 2007; Prinstein, Boergers, & Vernberg, 2001; Underwood, 2003). Nonetheless, because social aggression facilitates higher status in the peer hierarchy, children may be willing to use socially aggressive behaviours at the expense of being liked or accepted by their peers (Neal, 2010, p. 127). The positive feedback between increased social aggression and social status during middle childhood provides a strong motivation to engage in this form of social aggression. What is not clear is whether supportive relationships with teachers can disrupt this negative cycle of social aggression and peer rejection. Furthermore, limited research exists examining internal biological motivations to engage in socially aggressive behaviours. Similar to theories of physical aggression, emerging research in the neurobiology of social aggression suggests that children who are biologically under-aroused, may engage in socially aggressive forms of behaviour to increase their arousal to more comfortable states (Murray-Close et al., 2008). However, it is possible that specific subtypes of aggression, such as reactive, proactive or social aggression differ in their underlying neurobiological and social-contextual correlates in classroom settings.

**Prosocial behaviour.** In recent years, there has been a significant shift in how children and adolescents are viewed, with a move away from the traditional deficit-focused model of child development towards a strengths-based approach (Luthar & Brown, 2007; Masten & Motti-Stefanidi, 2008; Wentzel et al., 2007). The perspective underlying this shift is built around the knowledge that prosocial correlates such as empathy, altruism, and sharing, helping, behaviours are foundations of a healthy society (see work by Caprara, Barbaranelli, & Pastorelli,

2001; Wentzel, et al., 2007). Research suggests that prosocial children are rated as popular, friendly, and academically competent, whereas low social (neither prosocial nor antisocial) and antisocial children are often characterized as lonely, anxious, unpopular, and academically incompetent (Eisenberg, Fabes, & Spinrad, 2006; Wentzel & Erdley, 1993). Clearly, prosocial behaviours play an important role in promoting positive adaptation in the classroom setting. The focus on aggressive behaviours in the neurobiological literature however, has perhaps overshadowed the value of studying prosocial behaviours.

Prosocial behaviour, defined as voluntary behaviour intended to benefit another (Eisenberg, Fabes, & Spinrad, 2006; Staub, 1996), is a hallmark of social and emotional competence across childhood and adolescence. Due to the crucial role that prosocial behaviours play in forecasting children's positive development, understanding the factors associated with the development of children's prosocial behaviour has been an important goal for researchers interested in the promotion of competence and the prevention of maladjustment (e.g., Carlo, Mestre, Samper, Tur, & Armenta, 2010; Eisenberg & Eggum, 2008; Hardy, Carlo, & Roesch, 2010; Wentzel, Filisetti, & Looney, 2007). Numerous studies have demonstrated that children's prosocial behaviours such as cooperativeness, helpfulness, and comforting during middle childhood are related to friendships, social acceptance (Bukowski & Sippola, 1996; Wentzel & Erdley, 1993), and academic achievement (Caprara, Barbaranelli, Pastorelli, Bandura, & Zimbardo, 2000; Malecki & Elliot, 2002; Teo, Carlson, Mathieu, & Egeland, 1996; Wentzel, 1993; Wentzel & Caldwell, 1997). Prosocial behaviours in the classroom are thought to promote a social context that facilitates learning (Juvonen & Wentzel, 1996; Malecki & Elliot, 2002; Wentzel, 1991; Wentzel, 1993). In fact, peer- and teacher-rated prosocial behaviours during middle childhood are shown to be better predictors of grades than academic achievement scores (Wentzel & Erdley, 1993). With respect to gender, girls are generally shown to display higher prosocial behaviour compared to boys (Eisenberg, 2003; Eisenberg et al., 2006), although the

findings are inconsistent, with longitudinal research suggesting no gender difference in prosocial behaviours across middle childhood (Ma, Shek, & Tam, 2001).

Past research has found a developmental increase in children's prosocial behaviours across middle childhood (Eisenberg & Fabes, 1998; Eisenberg et al., 2006; Zahn-Waxler & Smith, 1992). In a series of longitudinal investigations by Eisenberg and colleagues, self-reports of empathy, sympathy, and prosocial behaviour during childhood have been associated with self-reported prosocial dispositions in young adulthood (Eisenberg et al., 1987; Eisenberg, Carlo, Murphy, & Van Court, 1995; Eisenberg et al., 1999; Eisenberg et al., 2002). Socialization processes as well as maturation in domains of empathy and socio-cognition (e.g., moral reasoning) are thought to underlie this increase in prosocial behaviours from childhood to adulthood (Eisenberg et al., 2006; Nantel-Vivier et al., 2009). Middle childhood appears to be a salient period in which to identify correlates of prosocial behaviours that are predictive of long-term adaptation. A fruitful area of research in school-age children appears to be the study of external contextual factors such as supportive relationships with peers and teachers (Hamre & Pianta, 2001; Wentzel, 1993; 2005; Wentzel et al., 2004; Wentzel, 2009). What is missing from the literature is a consideration of internal neurobiological processes as correlates of prosocial behaviour in middle childhood. In contrast to the burgeoning field of neurobiology and aggression, minimal research exists examining the association between HPA axis activity, or salivary cortisol, and children's prosocial behaviour. As will be discussed in later sections, theoretical evidence suggests that supportive peer and teacher relationships may influence the association between children's neurobiological functioning and prosocial behaviour.

### **Measurement of Social Behaviour in Middle Childhood**

Numerous methods exist to assess school-age children's social behaviours in classroom contexts. The four primary methodologies include: self-report (i.e., asking the children themselves about elements of social behaviour), peer assessments (i.e., asking children about

their perceptions of others within their peer group or classroom), adult reports (i.e., parents and teachers reports on children's social behaviours in the classroom), and observations (i.e., directly observing children during interactions with peers; see Pepler & Craig, 1998). Of these methodologies, peer assessment and teacher reports will be discussed with a particular focus on classroom-based research in elementary school-aged children.

There are a number of advantages of using peer assessments of prosocial and aggressive subtypes of behaviour in school settings (see Pepler & Craig, 1998, p. 177). For example, peers spend a great deal of time together, often away from adult supervision (e.g., playground, lunchroom). Therefore, peer assessments may be more accurate as there is greater opportunity for peers to witness or experience prosocial and aggressive behaviour. In addition, when peer assessments are obtained from a classroom of children, the multiple perspectives are thought to increase reliability and validity of the assessment, akin to multiple informants (Schonert-Reichl, 1999; Ladd, Herald-Brown, & Riser, 2008; Wentzel, Filisetti, & Looney, 2007). A common form of peer assessment of behaviour is the roster-and-rating peer nomination method (e.g., Ladd & Burgess, 2001; Parkhurst & Asher, 1992; Schonert-Reichl, 1999; Wentzel & Erdley, 1993). Peer nominations are obtained by giving students a list of names of their classmates and asking students to nominate classmates on characteristics (by circling name of classmate) specific to a subtype of behaviour (e.g., "shares and cooperates," prosocial behaviour). In general, students can circle as many or as few names as they want. A proportion score is calculated based on the percentage of nominations each student receives by dividing the number of nominations received by the total number of times their name appeared on nomination lists for a particular characteristic. Students with more nominations are considered to be higher on that characteristic. One disadvantage to using peer assessments however, is evidence that peer reports are relatively stable and as such, are not as likely to tap into measures of change in children's behaviour (e.g., following intervention; Pepler & Craig, 1998).

Teacher reports of classroom behaviour are typically assessed with a checklist in which teacher's judgments of an individual child are informed by that child's behaviour and interactions with peers (Pepler & Craig, 1998). Teachers generally have long-term and consistent experience with a large number of children during elementary school and are therefore able to judge children's behavioural development in relation to other children of similar age. However, teacher reports of aggressive behaviour in particular, may be unreliable given that aggression likely occurs beyond the eyes of the teacher. For example, evidence suggests that aggression occurs more frequently on the playground than in the classroom (Craig, Pepler, & Atlas, 2000). For this reason, multiple informants, such as peer nominations as well as teacher reports may provide a fuller picture of children's behavioural development in school contexts. In addition, the time of year in which children's behaviours are assessed appears to be significantly correlated with ratings of aggressive behaviour. For example, research in Grade 1 children suggests that teachers rate their class as more disruptive in the spring than in the previous fall semester (Conduct Problems Prevention Research Group (CPPRG), 1999). This phenomena is attributed to increased familiarity with students and thus increased ratings of misbehaviour, and the fact that children show increased aggression later in the year than earlier in the school term when children are often "on their best behaviour" (CPPRG, 1999, p. 655).

### **Correlates of Social Behaviour in Middle Childhood**

The average child will spend at least 15,000 hours in classrooms from kindergarten to high school during their education (Hamre, Pianta, & Chomat-Mooney, 2009). Therefore, research devoted to examining the nature of students' experiences in classrooms and the ways in which these experiences uniquely contribute to socio-emotional, behavioural, and physical development are of great importance (see Roeser, Eccles, & Sameroff, 2000 for a discussion on the importance of schools on social-emotional development). Classrooms offer a rich milieu in

which to examine basic developmental processes where each child is exposed to a social context (i.e., peer and teacher social interactions) over time (Mashburn et al., 2008; Myers & Pianta, 2008; Pianta, Belsky, Vandergrift, Houts, & Morrison, 2008). As such, the social climate of larger systems such as classrooms permits modeling of aspects of bio-ecological theory of development that emphasize the interplay between individual biological characteristics and the social-relational context (Bronfenbrenner, 2005; Mercer, McMillen, & DeRosier, 2009).

Methodological approaches for studying the antecedents and correlates of children's social behaviour in middle childhood have significantly lagged behind emerging perspectives of child development (Boyce et al., 2002). On the one hand, much has been learned regarding the importance of children's social relationships in predicting behaviour, in particular, the increasingly influential role of supportive relationships with peers and teachers during middle childhood (e.g., Brock, Nishida, Chiong, Grimm, & Rimm-Kaufman, 2008; Wentzel & Erdley, 1993; Wentzel, et al., 2007). On the other hand, researchers interested in the neurobiological correlates of social behaviour have provided evidence supporting an association between daily patterns in cortisol and children's behaviour (e.g., Adam, Klimes-Dougan, & Gunnar, 2007; Alink et al., 2008; Gunnar & Quevedo, 2007; van Goozen, Fairchild, Snoek, & Harold, 2007). Largely missing from both literatures however, are studies that examine the mediating influence of peer and teacher supportive relationships to obtain better insight into the processes by which cortisol and behaviour are linked. An integrative biology-by-context framework may provide a more complete model of child behavioural development. A central assumption underlying this framework is the recognition that children's lives are embedded in, and shaped by, multiple biological and social contexts that are fundamentally interdependent (Bronfenbrenner, 2005; Bronfenbrenner & Ceci, 1994). As such, this research aims to examine the mediating influence of peer and teacher supportive relationships on the association between daily patterns in cortisol and behaviour in school-age children in everyday classroom contexts. Importantly, the purpose

of this research is not to examine causal relationships per se, but rather to use mediation as a heuristic model to gain insight into the *processes* by which daily patterns in cortisol influence behaviour.

## **Summary**

Middle childhood is a time of great opportunity to optimize health and promote positive development. It is during the middle childhood years that children's personalities, behaviours, and competencies may consolidate into forms that likely will persist into adolescence and on into adulthood (Collins, 1984). Although there is much variation among children in terms of rate of growth and development, evidence is mounting that prosocial and aggressive behaviours during middle childhood are relatively stable, and are predictive of long-term adjustment and success (Huston, & Ripke, 2006). In particular, the emergence or consolidation of specific forms and functions of aggressive behaviours during middle childhood is shown to negatively impact children's socio-emotional functioning, academic success, and long-term adaptation (e.g., Coie et al., 1992; Kokko et al., 2006; Malecki & Elliot, 2002). In contrast, children's prosocial behaviours are associated with emotional competence, friendships, and academic achievement across middle childhood (e.g., Caprara et al., 2000; Caprara et al., 2001; Wentzel et al., 2007). For researchers interested in better understanding the antecedents of prosocial and aggressive behaviours in school contexts, studies show that peer and teacher reports provide unique perspectives of the different forms and functions of behaviour in middle childhood. The ability to identify qualitatively distinct forms and functions of behaviour has important theoretical value for our understanding of the development of childhood aggression, and represents a useful tool for identifying specific biological and contextual correlates in key social contexts, such as classrooms.

Consistent with a more integrative perspective of child development, the present study was designed to expand current understanding of the correlates and antecedents of children's social behaviour through an empirical examination of the mediating influence of peer and teacher supportive relationships on the association between daily patterns in cortisol and prosocial and aggressive subtypes of behaviour in middle childhood. Chapter 2 reviews some of the extant literature examining the role of supportive relationships with peers and teachers, and salivary cortisol as correlates of social behaviours in middle childhood.

## CHAPTER 2

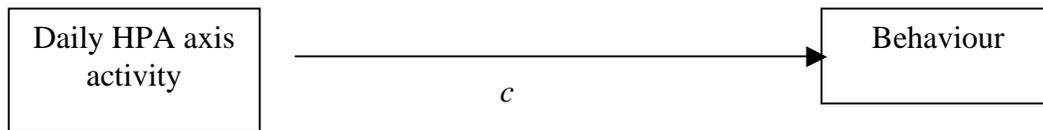
### Review of the Literature

The focus of this review is to integrate empirical and theoretical literature on the biological and social-contextual correlates of prosocial and aggressive behaviours in school-age children. The main goals of this research were to examine the associations between daily patterns of salivary cortisol and prosocial and aggressive behaviours, and to empirically investigate whether children's supportive relationships with peers and teachers mediate the association between cortisol and behaviour. To achieve these goals, four distinct bodies of literature are reviewed. The first section begins with a brief overview of the neurobiological system most often investigated in behavioural studies in middle childhood, the HPA axis and its end product, cortisol. The various factors that influence cortisol secretion will be presented (i.e., time of day, stressful events, puberty), followed by a review of the various indicators of daily patterns of salivary cortisol typically used in paediatric behavioural research (i.e., slope or change across the day). Next, the influence of early chronic stress on the developing HPA axis will be discussed as the basis for the proposal that children's daily HPA axis activity in middle childhood is a stable marker of earlier experience and significant correlate of behaviour. Building on this concept, empirical investigations examining the association between the HPA axis, indexed by daily patterns of salivary cortisol, and social behaviour during middle childhood are reviewed (Figure 1A, path *c*). Particular attention is given to the methodological inconsistencies found among these investigations that may help explain contradictory findings in the field. Next, evidence is presented showing that children's supportive relationships with peers and teachers are uniquely associated with children's behaviour in middle childhood (Figure 1B, path *b*). Situated within developmental theories of attachment and belonging, the distinct influence of peer acceptance and separately, teacher closeness on children's behavioural adjustment is reviewed. Then, evidence linking HPA axis activity in middle childhood to

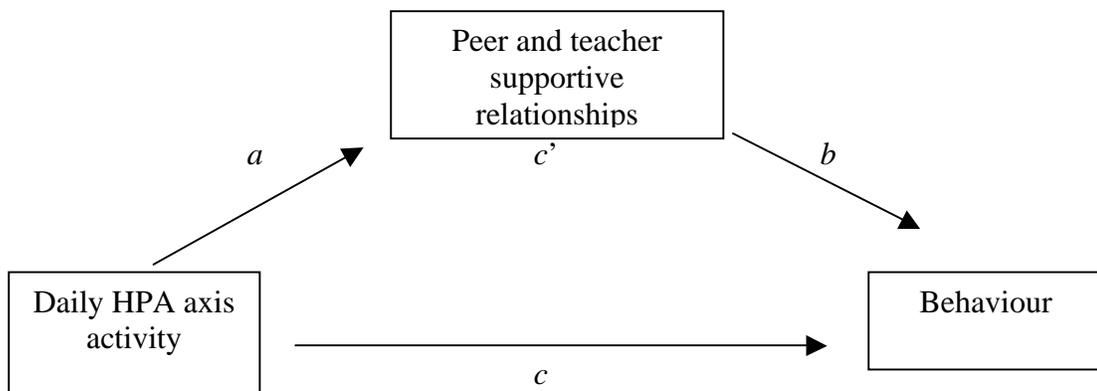
children's supportive relationships with peers and teachers is presented (Figure 1B, path *a*). Next, a unifying model describing peer and teacher supportive relationships as intervening, mediating variables in the association between children's daily patterns of cortisol and social behaviour is proposed (Figure 1B, path *c'*). Finally, the theory and concept underlying mediation analyses is introduced, and three common but conceptually distinct approaches to mediation analyses are reviewed and incorporated into the research design.

Figure 1. Simple mediation model. (A) Illustration of a direct effect, where daily HPA axis activity predicts behaviour. (B) Illustration of a mediation model where daily HPA axis activity exerts an indirect effect on behaviour through peer and teacher supportive relationships (adapted from Preacher & Hayes, 2008).

**A**



**B**



## **Neurobiology of the HPA Axis**

The brain stress response system is comprised of two main systems, the locus ceruleus/noradrenergic sympathetic system and the hypothalamic-pituitary-adrenocortical (HPA) axis (see Gunnar & Quevedo, 2007 for an in-depth overview of the neurobiology of stress in children). The HPA axis is the primary system of interest in children's developmental literature due to its established links with emotion-related brain circuitry (Shirtcliff et al., 2009), social affiliation (Taylor et al., 2008), and behaviour (see Gunnar & Quevedo, 2007; Lorber, 2004; van Goozen et al., 2007). The HPA axis is part of a larger neurobiological stress response system that functions to maintain a child's ability to respond to acute and prolonged changes in their environment. Although necessary for survival, chronic overstimulation of the HPA axis as a result of early chronic stress can lead to dysregulation of the HPA axis by middle childhood and short- and long-term physical, behavioural, and mental health problems (e.g., Brand et al., 2010; Cicchetti, Rogosch, Gunnar, & Toth, 2010; Gunnar, Frenn, Wewerka, & Van Ryzin, 2009; Matthews, 2002). Thus, investigations aimed at understanding the indirect, mediating processes by which HPA axis activity impacts behaviour during periods of critical development, such as middle childhood, have important implications for prevention and intervention efforts (e.g., Beauchaine, Neuhaus, Brenner, & Gatzke-Kopp, 2008; see Gunnar & Quevedo, 2007 for a discussion of the use of neurobiological markers in behavioural research; see also Cicchetti & Gunnar, 2008 for a special issue on integrating biology into intervention research).

Of the hormones released as part of the HPA axis, cortisol has received the most attention in the behavioural literature, probably due to the widespread regulatory influence of cortisol and ease of sampling with saliva. Cortisol plays a key role in multiple systems in the body including, but not limited to the central nervous system where it influences learning, memory, and emotion; the immune system where it regulates the inflammatory response; and the metabolic system where it regulates glucose storage and metabolism (see Miller, Chen, & Zhou, 2007). Moreover,

salivary measures are relatively easy and inexpensive to collect and hence are well-suited for population studies and paediatric research (see Adam & Kumari, 2009 for a review of assessing salivary cortisol in large-scale epidemiological research).

**Cortisol secretion.** In healthy individuals, cortisol has a known circadian rhythm and demonstrates a diurnal (light and dark) cycle. Cortisol is normally secreted in short bursts, with 15 to 30 pulses over the course of a day (King & Hegadoren, 2002). Cortisol levels rapidly increase immediately after awakening, producing a substantial (50% - 60%) increase in cortisol concentration within 20 - 45 minutes after waking (called the cortisol awakening response or CAR). As the day progresses, cortisol levels gradually decline and are lowest by midnight (Edwards, Clow, Evans, & Hucklebridge, 2001; Gunnar & Quevedo, 2007). Whilst levels in saliva are lower than in blood, salivary cortisol is strongly correlated with serum cortisol obtained from blood ( $r = 0.71- 0.96$ ) in healthy adults (Kirschbaum & Hellhammer, 1994) and pre-pubertal children (Bober et al., 1988) and are closely correlated with the “free” cortisol fraction or biologically active component of cortisol (Chrousos & Gold, 1992; Johnson, Kamilaris, Chrousos, & Gold, 1992).

When the brain perceives stress, the hypothalamus releases corticotropin-releasing factor (CRH) which triggers the release of adrenocorticotrophic hormone (ACTH) from the pituitary gland. The hypothalamus is an important area in the brain that connects the nervous system with the endocrine (hormonal) system via the pituitary gland (master brain hormone releasing gland; Chrousos & Gold, 1992). ACTH travels through the bloodstream and (along with signals from the brain sent through the autonomic nervous system) stimulates the adrenal glands to release cortisol (a stress hormone or glucocorticoid) and epinephrine (hormone or catecholamine) into the bloodstream (Chrousos & Gold, 1992). Cortisol and epinephrine help provide energy, oxygen, and stimulation to the heart, brain, and other muscles and organs to support the body's response to stress. There is an estimated lapse of 15 to 30 minutes between a stressful event and

the production and release of cortisol into the bloodstream (i.e., plasma cortisol), with an additional 2 minute lapse before cortisol increases in saliva (Gunnar & White, 2001). Under typical conditions, when the brain perceives that the stress has ended, negative feedback signals the return of hormone levels to baseline values (de Kloet, 2000; Sapolsky, Romero, & Munck, 2000). Release of CRH from the hypothalamus is influenced by a number of factors, including circulating blood levels of cortisol, the sleep/wake cycle, and stressful events such as physical (e.g., exercise) or social (e.g., public speaking) challenges. Circulating basal levels of cortisol have fast-acting effects on the brain as well as long-lasting effects on genomic alterations (de Kloet, 2004; Sapolsky et al., 2000; Shirtcliff et al., 2009). In addition to the baseline circadian cycle, cortisol is also secreted in response to an acute stressor (e.g., experimentally-induced social stressor) showing rapid increases in cortisol levels 20 minutes post-stressor. Short-term cortisol release in response to stress serves an adaptive function, however prolonged exposure to chronic stress (i.e., early adverse rearing environments) may result in dysregulation of the HPA axis, possibly due to overstimulation and desensitization of brain cortisol (glucocorticoid) receptors, predisposing children to physical and mental health problems (Cicchetti, et al., 2010; Matthews, 2002; McEwen, 2000). Thus, whether elevations or suppressions in HPA axis activity are observed depends on the time of day and the time since onset of chronic stress (see Miller et al., 2007 for a review of chronic stress and the HPA axis). Additionally, cortisol is influenced by a number of individual and external factors that will be discussed below.

**Factors influencing daily cortisol secretion.** Daily cortisol is highly variable, therefore the factors that influence children's daily cortisol secretion need to be taken into consideration in the design of research investigations (Gunnar & Quevedo, 2007, p. 519; see Hanrahan, McCarthy, Kleiber, Lutgendorf, & Tsalikian, 2006, p. 96 for a discussion of salivary cortisol collection in paediatric research). Salivary cortisol is influenced by time of day, sleep, certain medications (e.g., corticosteroids), certain illnesses (e.g., Cushing's syndrome), and the use of

salivary stimulants (see Granger et al., 2007; Hanrahan et al., 2006; Hibel, Granger, Cicchetti, & Rogosch, 2007). Evidence suggests that exercise and eating may cause transient changes in cortisol levels (Kirschbaum & Hellhammer, 1994), while changes in sleeping patterns, waking times, and travel across time-zones may alter the diurnal rhythm of cortisol (e.g., El-Sheikh, Buckhalt, Keller, & Granger, 2008; King & Hegadoren, 2002; Jessop & Turner-Cobb, 2008; Matchock, Dorn, & Susman, 2007; Vreeburg et al., 2009). In adults, research shows that gender, smoking, physical activity, and months with daylight significantly influence daily patterns in salivary cortisol obtained in a home setting (Vreeburg et al., 2009). Individual factors such as age, body mass index (BMI), pubertal stage, and gender are shown to influence cortisol variability in children (see Gunnar & White, 2001; Jessop & Turner-Cobb, 2008; Oskis, Loveday, Hucklebrdige, Thorn, & Clow, 2009). For example, cross-sectional research on adolescent females (9 -18 years) suggests that older adolescents girls who have undergone menarche (post-menarche) have higher cortisol levels across the day compared to pre-menarche girls. Cortisol follows a circadian rhythm; therefore time of day and time since awakening are important factors in studies examining daily patterns in salivary cortisol. In fact, research in a large sample of adolescents ( $N = 2,995$ ) has shown that time of morning cortisol sampling, and time since awakening were the most influential confounders on two samples of morning (9am) salivary cortisol obtained in a naturalistic, school setting (Kelly et al., 2008). In addition to sampling in the morning, some researchers suggest that sampling cortisol in the afternoon is preferable as there is less variation in cortisol secretion in the afternoon (e.g., Jessop & Turner-Cobb, 2008; Smider et al., 2002).

The behavioural literature is complicated by the use of diverse settings to obtain samples of salivary cortisol from children (e.g., home, school, or laboratory). In adults, cortisol sampling on a work day compared to a weekend results in higher cortisol concentrations upon awakening, perhaps as a result of anticipatory stress before going to work (Vreeburg et al., 2009). Research

in young children suggests that a secondary increase in afternoon cortisol is observed in children attending day care compared to home settings (see Vermeer & van IJzendoorn, 2006 for a review and meta-analysis). Importantly, variation in the quality of day care is significantly associated with these cortisol increases suggesting that daily patterns in cortisol are also influenced by the quality of relationships with caregivers (Watamura, Kryzer, & Robertson, 2009). This association between social context and daily patterns of cortisol will be explored in further detail later on in this review.

**Indicators of daily patterns of salivary cortisol.** The dynamics of daily cortisol production yield many different types of cortisol measures (e.g., basal secretion, peak secretion, pulse amplitude and frequency) and many ways in which children can differ in their daily patterns of cortisol (e.g., single point-in-time estimate, change across the day, total daily output, global daily average; Gunnar & Quevedo, 2007). Recent research is starting to unravel the exact meaning and relevance of each of these elements of daily HPA axis functioning in relation to health and disease (see Adam & Kumari, 2009; Chida & Steptoe, 2009). However, a consequence of this multiplicity in measurement is the lack of consistency in terminology used among investigators to characterize indicators of daily patterns in cortisol (e.g., cortisol levels, diurnal change, average cortisol, cortisol output). To facilitate a better understanding of the terminology used within these studies, the following section will briefly review the various indicators of daily patterns in salivary cortisol used in the child behavioural literature and the language used to characterize them (i.e., point-in-time, average levels, global average). The term “daily patterns in salivary cortisol” is used throughout this review, not to reflect a dynamic measurement model as ‘patterns’ may suggest, but in a narrower sense to represent various measures of salivary cortisol obtained under resting conditions in a non-experimentally stimulated setting (e.g., cortisol levels, diurnal change, average cortisol, cortisol output).

***Cortisol at one specific time point across the waking day.*** The concentration of salivary cortisol obtained either at a specific clock time (e.g., 3pm) or time point during the day (e.g., afternoon) represents a single point-in-time estimate of cortisol concentration. Single point-in-time estimates are unreliable unless the time of awakening is known (e.g., Adam, 2006; Adam & Kumari, 2009). Researchers using a single sample of salivary cortisol in relation to aggressive behaviour in children have described this measure as an indication of “basal cortisol concentrations” or “cortisol levels” (e.g., Loney, Butler, Lima, Counts, & Eckel, 2006; Oosterlaan, Guerts, Knol, & Sergeant, 2005). A more accurate characterization would be to define this measure in relation to the number of hours elapsed since the child awoke (e.g., 7.5 hours post-awakening cortisol concentration). However, with the advent of inexpensive and more informative multiple sampling techniques, single point-in-time estimates of cortisol will likely be replaced by multiple daily sampling of cortisol in future behavioural investigations (e.g., Suglia, Staudenmayer, Cohen, & Wright, 2010).

***Average morning/afternoon/evening cortisol levels.*** A number of studies have employed the term “average cortisol levels” to represent an average of more than one cortisol sample taken at a similar time of day. For example, some researchers have sampled salivary cortisol either in the morning (e.g., Lupien, King, Meaney, & McEwen, 2001), afternoon (e.g., Popma et al., 2007b; Smider et al., 2002; Tyrka et al., 2010) or evening (e.g., Vreeburg et al., 2009), using terminology such as “basal cortisol levels,” “afternoon cortisol levels,” and “evening cortisol,” respectively. Some researchers claim that sampling salivary cortisol in the afternoon is preferable to morning as afternoon reflects a relatively quiescent period of cortisol release compared with morning hours (Jessop & Turner-Cobb, 2008) and consequently minimizes the extent of within-person variability (Smider et al., 2002). Furthermore, research suggests that morning and afternoon cortisol levels offer information about both intrinsic biological processes (i.e., diurnal rhythms) as well as external contextual processes such as social and emotional

events (see Shirtcliff & Essex, 2008). Specifically, it is suggested that morning levels are influenced by unique factors that are largely genetic (Bartels et al., 2003), whereas afternoon levels are subject to less genetic influence and are more easily influenced by the immediate social context (Schreiber et al., 2006).

***Global daily average.*** Some researchers have calculated the average of two or more daily cortisol samples taken at *different* times of the day from youth (e.g., morning and afternoon) and termed these averages “mean cortisol levels” (e.g., Weems & Carrion, 2007). This method of combining both morning and afternoon levels is perhaps better represented by the term, “global daily average.” A significant limitation of this indicator is that it does not take into consideration specific aspects of the cortisol diurnal rhythm, potentially masking any differences attributed to morning or afternoon cortisol concentration.

***Diurnal cortisol slope.*** A number of investigations use diurnal cortisol slope as an indicator of the “degree of change (typically decline) in cortisol levels from early morning to late evening” (Adam & Kumari, 2009, p. 1425). Diurnal slope can be calculated by subtracting the value at the end of the sampling time frame (e.g., afternoon/evening) from the first sample (e.g., morning) and dividing it by the number of hours between the two samples (Vreeburg et al., 2009). Ideally, each child’s time since awakening should be taken into consideration when calculating indicators of daily cortisol such as slope due to the circadian nature of cortisol secretion (see work by Miller et al., 2007; Suglia et al., 2010). Common terminology found within the paediatric behavioural literature to describe diurnal cortisol slope includes; “change in cortisol over the day,” “cortisol levels over the day,” and “basal cortisol decline/rise” (see Gunnar, Kryzer, VanRyzin, & Phillips, 2010). Research shows that children’s diurnal cortisol slope is significantly associated with early chronic stress (see Gunnar & Quevedo, 2007), mental health symptoms (e.g., Shirtcliff and Essex, 2008), aggressive behaviours (e.g., Murray-Close et al., 2008), and environmental context (e.g., Watamura, Donzella, Alwin, & Gunnar, 2003). Evidence

suggests that a flatter decline in cortisol slope is typically associated with early chronic stress in young children (Gunnar & Vazquez, 2001) and mental health problems in middle childhood. For example, Shirtcliff and Essex (2008) recently measured salivary cortisol at three discrete times across the day (i.e., upon awakening, late afternoon, evening) and found that low “basal cortisol levels” as well as a flatter “cortisol slope” predicted more severe mental health symptoms in 5<sup>th</sup> grade children. Moreover, flatter cortisol slopes were associated with mental health symptoms at both 5<sup>th</sup> and 7<sup>th</sup> grade. The authors suggested that alterations in daily rhythm are better predictors of mental health symptoms than absolute levels of cortisol.

*Area under the daytime cortisol curve.* Another popular indicator of salivary cortisol is called area under the curve (AUC) based on the trapezoid formula used to compute this measure (see Pruessner, Kirschbaum, Meinischmid, & Hellhammer, 2003). Research investigations with repeated sampling of daily cortisol may compute AUC cortisol as a measure of the “average cortisol exposure,” which does not provide an indication of diurnal change (Adam & Kumari, 2009). AUC can be used as an indicator of the cortisol awakening response (CAR), diurnal cortisol measured across the waking day, or cortisol response to an experimentally induced stressor. Two formulas have been proposed for AUC that are thought to capture the “intensity” as well as the “sensitivity” of repeated measures of cortisol (Pruessner et al., 2003; Fekedulegn et al., 2007). Area under the curve with respect to ground (AUC<sub>g</sub>) is an estimate of the intensity of the cortisol response, or the “total cortisol concentration” secreted within a specific time frame of multiple samples. Area under the curve with respect to the increase in cortisol (AUC<sub>i</sub>) is defined as the area under the curve above the baseline value (typically the value of the first cortisol sample) minus the area above the curve below the baseline value. AUC<sub>i</sub> is a measure of sensitivity of the cortisol response, or the rate of change of repeated measurements over time (Fekedulegn et al., 2007). Longitudinal research examining daily patterns of cortisol have shown that adolescents at risk for psychosis demonstrate higher morning (9am) AUC<sub>i</sub> and AUC<sub>g</sub>

cortisol compared to adolescents who do not convert to psychosis after one year (Walker et al., 2010). The cortisol awakening response or CAR represents the size of the post-awakening increase in cortisol that occurs within 30 - 45 minutes after waking. Both heightened and blunted CARs have been associated with psychosocial stress and poor health outcomes (see Chida & Steptoe, 2009 for a review). However, in adolescents no significant difference is observed in the CAR between adolescents with conduct disorder (CD) and comparison groups, although higher evening cortisol was found in CD groups in one evening cortisol sample (11pm) obtained at home (Fairchild et al., 2008).

***Multiple indicators of daily patterns of salivary cortisol.*** Some investigators have sampled cortisol at multiple times across the day to yield a variety of cortisol indicators for use within their analyses. For example, Suglia and colleagues (2010) collected salivary cortisol samples from children in a home setting, four times a day (upon awakening, before lunch, before dinner and at bedtime) over three days. Results demonstrated that children living in stressful communities with posttraumatic stress disorder (PTSD) symptoms (e.g., arousal, intrusion, and avoidance) had “elevated afternoon and evening cortisol levels”, also represented by greater AUC cortisol and “blunted diurnal slope” (e.g., Suglia et al., 2010). All analyses controlled for time since awakening, race/ethnicity, SES, age, and gender. In another example, Sondejker et al. (2008) obtained two morning samples and one evening sample from school-aged Dutch children (aged 10 to 12 years) at home, before, and after a regular school day to obtain a measure of “basal cortisol levels”. Two indicators of cortisol were used in their analyses: evening cortisol, and AUC for their two morning samples as a measure of “morning cortisol concentration”. Results of their analyses suggested that “lower evening cortisol levels” significantly predicted parent-reported disruptive behaviours two years later in boys who already scored high on disruptive problems. In another study, Popma et al. (2007a) examined the relationship between the “diurnal cortisol cycle”, as well as the cortisol awakening response to antisocial behavior in

male adolescents. Results suggested that boys diagnosed with disruptive behaviour disorder (DP) demonstrated a “flatter cortisol slope” and “lower cortisol levels” in the first hour after awakening (smaller CAR) compared to healthy controls. Thus, it would appear that multiple sampling of daily cortisol across numerous days to obtain various indicators of daily patterns in salivary cortisol (e.g., evening levels, AUC, or slope) might offer more information on how cortisol and behaviour are linked, compared to single samples of cortisol. Furthermore, studies in which multiple assessments of cortisol are taken over time provide some evidence for short-term stability in measures of cortisol in infancy and childhood, although some degree of variation is also evident (see Doussard-Roosevelt, Montgomery, & Porges, 2003). For example, recent research in young adults has shown that basal measures of salivary cortisol are stable in both men and women across a two-week period (Liening, Stanton, Saini, & Schultheiss, 2010).

### **Summary of the Neurobiology of the HPA Axis**

The HPA axis is a key system of interest in behavioural research due to its role in the human stress response and ease of sampling cortisol in saliva. Cortisol production demonstrates a diurnal rhythm, showing highest levels in the morning, gradually decreasing over the day (Edwards et al., 2001). However, whether elevations or suppressions in HPA axis activity are observed also depends on various individual and environmental factors such as pubertal stage, time of day, and time since the onset of acute and chronic stressors (Gunnar & Quevedo, 2007). Researchers have devised a number of different indicators of daily patterns in cortisol to index HPA axis functioning. However, there appears to be a lack of consistency in methodology and terminology used among investigators to characterize indicators of daily patterns in cortisol. Ideally, sampling of cortisol should provide at least some estimate of the key diurnal cortisol parameters such as the decline in cortisol across the day (slope) or average level of cortisol at a specific time during the day (average morning or afternoon). Factors that influence children’s daily cortisol secretion, especially time of day, and time since awakening need to be taken into

consideration in behavioural research studies. Based on these findings, the current study was designed to sample multiple measures of daily cortisol across several days to provide various indicators of daily patterns in salivary cortisol including average morning, noon, and afternoon levels, area under the daytime cortisol curve, and diurnal slope.

### **The Impact of Chronic Early Stress on the Developing HPA axis**

A review of some of the existing developmental literature suggests that the HPA axis is programmed during critical periods of development in infancy and childhood, establishing a particular trajectory of functioning over the life course (e.g., Bauer & Boyce, 2004; Boyce & Ellis, 2005; see Gunnar & Quevedo, 2007; Ryff & Singer, 2000; Seeman & McEwen, 1996). The impact of chronic HPA axis activation within the developing brain is of particular interest during childhood, helping to explain how early rearing experiences (e.g., Heim & Nemeroff, 1999; Sánchez, Ladd, & Plotsky, 2001; Tarullo & Gunnar, 2006) may result in dysregulation of the HPA axis that subsequently increases the risk of behavioural problems by middle childhood (Alink et al., 2008; Matthews, 2002; Weinstock, 2008). The following section will introduce theoretical and empirical evidence demonstrating a significant association between early chronic stress and dysregulation of HPA axis functioning in middle childhood.

**Chronic early stress and HPA axis dysregulation.** One common explanation by which early adverse environments are thought to shape HPA axis functioning is through frequent elevations in cortisol that over time may result in down-regulation of the HPA axis, resulting in low rather than high cortisol secretion later in life and a flattening of the cortisol diurnal slope (Gunnar & Quevedo, 2007). However, the literature is complicated by evidence that the nature and timing of chronic stress may have differential effects on HPA axis functioning. Evidence suggests that the HPA axis tends to hyperactivate in response to an acute stressor, eventually down regulating in response to hyperactivation. Thus, in response to chronic stressors, the HPA

axis may show hyper- and, later, hypo-activity (see Miller et al., 2007). For example, young foster children (age 3 to 6 years) with a history of maltreatment and physical neglect demonstrate lower average “morning cortisol levels” (two salivary samples obtained 30 minutes after awakening in a home setting) compared to non-maltreated children (Bruce, Fisher, Pears, & Levine, 2009). Whereas foster children with severe emotional maltreatment demonstrate “high morning cortisol levels” compared to non-maltreated children. The authors suggest that physical abuse is representative of a chronically stressful environment resulting in lower cortisol, whereas emotional maltreatment represents an acute stressor, resulting in higher cortisol (see Fries, Ziegler, Kurian, Jacoris, & Pollak, 2005, see also Wismer Fries, Shirtcliff, & Pollak, 2009). This interpretation is consistent with findings from a recent meta-analysis examining chronic stress in adult populations where it is suggested that the time of onset of chronic stress is negatively associated with HPA axis activity. That is, the more time that has elapsed since the onset of a stressor, the lower an individual’s morning cortisol, and daily volume. Whereas, if chronic stressors are still present in an individual’s environment (e.g., unemployment), then morning, afternoon/evening, and daily cortisol output are significantly higher (Miller et al., 2007, p. 35). Miller and colleagues suggest that this time-dependent pattern of HPA axis activity may be responsible for the contradictory findings in the field linking stress and behaviour to either high or low cortisol. However, rather than being contradictory, it is suggested that these depictions of HPA axis activity are accurate and reflect time-sensitive aspects of the HPA axis in response to stress. Consistent with the recent proposal put forth by Cicchetti et al. (2010), a more accurate conceptualization of high or low HPA axis activity appears to be the term “HPA axis dysregulation”. As such, the concept of HPA dysregulation will be used throughout the remainder of this dissertation to represent HPA axis activity and daily patterns of cortisol that differ from typical circadian patterns of cortisol.

**HPA axis activity in middle childhood.** The influence of early adverse environments on HPA axis functioning is shown to persist over the long-term into middle childhood (Cicchetti et al., 2010) and even adulthood (Luecken, Kraft, & Hagan, 2009). Cross-sectional studies have shown that children from families with low socio-economic status or children living with a history of maltreatment demonstrate HPA axis dysregulation in middle childhood, such as higher morning and evening cortisol (Gustafsson, Gustafsson, & Nelson, 2006). For example, Lupien et al., (2000, 2001) observed elevated “basal morning cortisol” in children (aged 6 – 10 years) from families with low compared to high socio-economic status. The investigators used the average of two morning basal salivary cortisol samples (both obtained around 8am) from children in a classroom setting. More recently, Cicchetti and colleagues (2010) found that school-aged children exhibiting internalizing symptoms (e.g., depression) who had experienced physical or sexual abuse in the first five years of life displayed “atypical flattening of basal cortisol production” while attending a week-long day camp. The blunted diurnal slope represented by a slight lowering of morning and a slight elevation of afternoon and evening basal cortisol levels, were thought to reflect persistently low basal cortisol in response to chronic stress. The observed flatter diurnal slope in children from chronically stressful environments is consistent with results obtained by Gunnar and Quevedo (2007) in their review of HPA axis functioning in studies of younger children.

A potential implication of dysregulation of HPA axis functioning in middle childhood is an increased risk of emotional and behavioural problems. However, the association between HPA axis functioning and behaviour is not limited to children with a history of chronic stress. A number of cross-sectional and longitudinal investigations have shown that HPA axis dysregulation is associated with, and predictive of, emotional and behavioural problems in community samples of children (e.g., El-Sheik, Erath, Buckhalt, Granger, & Mize, 2008; Murray-Close et al., 2008; Shirtcliff & Essex, 2008; Shirtcliff, Granger, Booth, & Johnson,

2005; Smider et al., 2002). These studies are based on the premise that daily HPA axis activity by middle childhood is a stable marker of early experience, and not just in the case of severe adverse rearing environments. In fact, evidence suggests that the association between daily patterns in cortisol (i.e., blunted and steeper diurnal slope) and specific subtypes of aggression are more pronounced for children with no history of maltreatment compared to maltreated children (Murray-Close et al., 2008). One explanation for this finding is that biological contributors to involvement in aggressive behaviours may be more influential in children who have fewer risk factors for aggression (Murray-Close et al., 2008, p. 14; Raine & Venables, 1981). Building from this theoretical foundation, the following section will review studies examining the role of daily patterns of salivary cortisol and behaviour during middle childhood in both clinical and non-clinical populations.

### **Summary of the Impact of Chronic Early Stress on the Developing HPA Axis**

Evidence suggests that chronic stimulation of the HPA axis during childhood may result in dysregulation of the HPA axis by middle childhood that subsequently increases the risk of short- and long-term emotional, behavioural, and mental health problems (e.g., Alink et al., 2008; Cicchetti et al., 2010; Heim & Nemeroff, 1999; Sánchez, et al., 2001; Tarullo & Gunnar, 2006; Weinstock, 2008). Of particular interest is the evidence that dysregulation of HPA axis is not limited to children with a history of maltreatment or abuse, but may be more pronounced in community sampled children who have fewer risk factors for aggression (e.g., El-Sheik et al., 2008; Murray-Close et al., 2008; Shirtcliff & Essex, 2008; Smider et al., 2002). What is missing from the literature are studies examining the association between HPA axis and prosocial and aggressive subtypes of behaviour in typically developing children in everyday classroom contexts. Research of this nature may help provide a better understanding of the biological profiles of individual children at risk for aggression, or conversely, those prosocial children thriving in classroom contexts.

## **Daily Patterns of Salivary Cortisol and Behaviour in Middle Childhood (path *c*)**

Healthy HPA axis functioning is thought to require the presence of strong diurnal patterning in cortisol. Deviations from strong diurnal patterning of the HPA axis may provide valuable information about the role of the HPA axis in the development of children's behaviour in middle childhood (see Adam & Kumari, 2009; Alink et al., 2008; Matthews, 2002; Weinstock, 2008). During the past decade, there has been a burgeoning literature examining the link between daily patterns of salivary cortisol and aggressive behaviours in children (see Adam, Klimes-Dougan, & Gunnar, 2007; Alink et al., 2008; Gunnar & Quevedo, 2007; Granger & Kivlighan, 2003; Granger et al., 2007; Jessop & Turner-Cobb, 2008; van Goozen et al., 2007 for reviews). However, the findings from these investigations have been equivocal and sometimes contradictory. A review of some of the extant literature reveals a wide range in methodologies that likely contribute to the inconsistent findings in the field, including the use of different indicators of salivary cortisol (e.g., average levels, diurnal slope), measured in different contexts (e.g., home, day care, laboratory), with limited attention to specific subtypes of aggression (e.g., social, proactive, reactive). Missing from this research is an examination of the specific aspects of cortisol diurnal patterning (e.g., morning levels, afternoon levels, or change over the day) that are related to subtypes of aggression in children residing in everyday contexts such as classrooms (e.g., Shirtcliff & Essex, 2008). Additionally, there is a need to examine the potential role that other factors, such as supportive relationships (peer acceptance and teacher closeness), may play in mediating the relation between daily cortisol patterns and children's social behaviours. Research of this nature may serve as a first step toward understanding the processes by which daily patterns in cortisol influence behaviour in typically developing children in everyday classroom environments. The following section reviews findings from a number of investigations in school-age children examining the association between salivary cortisol and aggressive behaviours. Next, research examining the association between salivary cortisol and

reactive, proactive, and social aggression will be discussed. At the time of this review, there was a notable deficit of peer-reviewed investigations examining the association between daily patterns in cortisol and prosocial behaviours in middle childhood.

**HPA axis and aggressive behaviours.** Many studies have examined salivary cortisol levels in response to a stressful laboratory challenge in clinical cohorts of children, (Oosterlaan et al., 2005; Pajer, Gardner, Rubin, Perel, & Neal, 2001; Popma et al., 2007b; Randazzo, Dockray, & Susman, 2008). However, caution should be taken when interpreting results from these studies and comparing them to investigations examining children from non-clinical, typical populations. Evidence suggests that children diagnosed with clinical behavioural problems differ significantly in their stress response functioning from non-clinical populations (Loney et al., 2006; Popma et al., 2007a; van Goozen, et al., 1998). Of the research examining daily patterns in salivary cortisol, some investigations have revealed little or no relationship between cortisol and concurrent behavioural problems in children (e.g., Kruesi, Schmidt, Donnelly, & Hibbs, 1989; Sondejker et al., 2007). However, several investigations have found a positive association between daily patterns in cortisol and externalizing behavioural problems in healthy (El-Sheikh, Erath, & Buckhalt et al., 2008; Gustafsson, Gustafsson, & Nelson, 2006; Klimes-Dougan, Hastings, Granger, Usher, & Zahn-Waxler, 2001; Susman et al., 2007; Tyrka et al., in press) and clinical populations of children and adolescents (Popma et al., 2007b; van Bokhoven et al., 2005). Externalizing behaviour problems such as disruptive, hyperactive, and aggressive behaviours are a group of outward displays of behaviour where the child is negatively acting on the external environment (Campbell, Shaw, & Gillion, 2000). Of the studies finding a positive association between cortisol and externalizing, aggressive behaviours, investigators have also used a variety of different indicators of salivary cortisol and methodologies. For example, El-Sheik, Erath, Buckhalt et al. (2008) calculated “basal cortisol” as an average of two cortisol samples from children (aged 8 to 9) in a laboratory setting prior to, and following a stress

challenge at an indeterminate time of day (average time of day was 2pm). Although time of sampling was considered in the analyses, the cortisol samples may have been influenced by the anticipation and stimulation of the experimental session and probably do not represent cortisol patterns obtained in a naturalistic setting. One explanation for the observed positive association between cortisol levels and externalizing, aggressive behaviour is offered by El-Sheik, Erath, Buckhalt and colleagues (2008) who suggest that increased HPA axis activity may prime social information processing biases associated with aggression (Mize & Pettit, 2008) such as increased attention to perceived threat (Vasey & Daleiden, 1996).

In another study, van Bokhoven and colleagues (2005) obtained one sample of morning (9am) salivary cortisol, “baseline cortisol level,” from 13 year old boys upon arrival to a laboratory setting. The authors observed that “high levels of cortisol” at age 13 were positively associated with reactive aggression (average score of reactive aggression over four years, age 12 to 15 years) and conduct disorder (measured two years later at age 14 to 16 years). The investigators did not control for influential factors such as time of awakening, medications, diet, illness or pubertal status. Similar results were obtained in an earlier study where Smider and colleagues (2002) obtained afternoon cortisol samples (between 3pm and 7pm) from children (aged 4.5 years) in a home setting on three consecutive days. The average of all three samples was calculated to give an indicator of “average afternoon cortisol levels.” Their findings suggested that higher average afternoon cortisol levels predicted higher ratings of father-reported externalizing behaviours in boys a year and a half later. Notably, afternoon cortisol levels were not significantly associated with behaviour measured concurrently with home afternoon cortisol. What is notable about these two longitudinal studies is the finding that patterns of daily cortisol were predictive of long-term behavioural problems supporting the theoretical perspective that HPA axis dysregulation in middle childhood may predict or precede behavioural development.

In contrast to studies observing a positive association between cortisol and aggression, a number of researchers have found an inverse association between low daily cortisol and externalizing behaviour problems in children (see Alink et al., 2008 for a review; e.g., Loney et al., 2006; McBurnett, Lahey, Rathouz, & Loeber, 2000; Oosterlaan et al., 2005; Pajer et al., 2001; Shirtcliff, Granger, Booth, & Johnson, 2005; Shoal, Giancola, & Kirillova, 2003). However, similar to those studies described above, these investigations utilized a variety of different indicators of salivary cortisol across different contexts and populations that question the generalizability of the findings. For example, Popma et al. (2007a) found that boys diagnosed with disruptive behaviour disorder demonstrate a “flatter cortisol slope” and “lower cortisol levels” in the first hour after awakening (smaller CAR) compared to healthy controls. Cortisol levels in the afternoon or evening (home sample obtained on one day only) were found not to significantly differ among healthy boys or boys with disruptive behaviour disorder. However, the afternoon samples were collected during a laboratory visit after school (mean of two samples collected within one hour of each other) that may have led to extraneously high values due to the novelty of a laboratory environment. In another study, Loney and colleagues (2006) obtained one sample of morning (9am) salivary cortisol from adolescents in a school setting. Their findings suggest that adolescent males characterized by high callous-unemotional traits (e.g., lack of guilt and empathy) have lower “resting cortisol levels.” There was no mention of controlling for factors that influence cortisol secretion such as time of awakening, medications, diet, illness, or pubertal status. In a similar design using only a single point-in-time estimate of daily cortisol, Oosterlaan et al. (2005) found that that lower “basal cortisol levels” were associated with increased ratings of teacher-reported conduct disorder in children aged 6 to 12 years. However, the single cortisol sample was obtained in a laboratory setting from children at different times of day (between the hours of 10am and 3pm) in a small sample with no mention of controlling for diet, food, exercise, or time since awakening.

A common explanation for the association between low HPA axis activity, or low cortisol, and aggressive behaviours is attributed to the proposal that cortisol mediates an inhibited, fearful, or anxious state. Thus, low levels of cortisol may signal biological under-arousal contributing to stimulation-seeking or fearlessness, which in turn makes aggression more likely (e.g., van Goozen, Matthys, Kettenis, Buitelaar, & Engeland, 2000; Raine, 1996). Moreover, those children who often seek stimulation may be involved in frequent stressful situations and eventually habituate to these stimuli and show a blunted stress response or low basal cortisol levels (Alink et al., 2008; van Goozen et al., 2007). In effect, behaviours such as aggression may provide under-aroused children with stimulation that increases their cortisol levels to more comfortable levels (Murray-Close et al., 2008). Fearlessness has also been associated with low cortisol levels and aggression due to a lack of inhibition of being involved in aggressive behaviours (Raine, 2002). Consistent with both perspectives (see Murray-Close et al., 2008), emerging research suggests that low cortisol is associated with elevated sensation seeking in adult males (Rosenblitt, Soler, Johnson, & Quadagno, 2001) and impaired fear reactivity in young children (Kagan, Reznick, & Snidman, & Gibbons, 1988).

Studies that measure cortisol levels at multiple sampling points, rather than at one time point, may offer greater consistency in results (e.g., Klimes-Dougan et al., 2001). Emerging research has observed a move towards studying daily patterns in resting cortisol levels to better understand the neurobiological correlates of emotional and behavioural development in middle childhood (Adam, 2006; Cicchetti, et al., 2010). For example, Murray-Close et al. (2008) examined the association between daily cortisol patterns and aggression using the average of three cortisol samples (morning, pre-lunch, and afternoon) over 5 days obtained from school-age children (6 to 12 years) while attending a summer day camp. They found that children displaying “lower levels of cortisol” following the (9am) morning arrival and a more gradual decline in cortisol over the day were rated higher on measures of relational aggression by peers and camp

counsellors, whereas children displaying steeper declines in cortisol over the day were rated higher on measures of physical aggression. These results suggest that specific patterns of daily cortisol production are associated with specific subtypes of aggression. However, these findings were limited to a week long summer day camp where peers and counsellors were relatively unfamiliar with one another.

Consistent with the terminology proposed earlier, what appears to be important in studies of cortisol and aggression is not whether there is high or low activity of the HPA axis or daily patterns in cortisol, but whether there is dysregulation of the HPA axis. Importantly, as discussed in the previous chapter, children may engage in a variety of different forms of aggressive behaviour (e.g., physical and relational) and functions (e.g., proactive and reactive), that are each related to short- and long-term adjustment in different ways (Little et al., 2003; Murray-Close & Ostrov, 2009). Only in recent years, have investigators started to examine daily patterns in cortisol in relation to specific subtypes of childhood aggression. However, the majority of these investigations appear to be limited by the methodological inconsistencies described above.

***Reactive and proactive aggression.*** Previous research examining HPA axis activity and proactive and reactive subtypes of aggressive behaviour in children has focused primarily on stress reactivity (i.e., acute activation of the HPA axis in response to an experimentally induced social challenge). Of these investigations, the common inference is that an over-active HPA axis response to an acute stressor is associated with reactive, but not proactive aggression. This is based on the theoretical perspective that the two subtypes of aggression have fundamentally different motivations. Reactive aggression is based on the frustration-aggression model which suggests that aggression is a hostile and angry retaliation to frustration (Berkowitz, 1989), whereas proactive aggression is associated with fearlessness and stimulation-seeking associated with low basal cortisol. Thus, reactive but not proactive aggression is thought to relate to acute increases in biological arousal during stressful events (Hubbard et al., 2002). Emerging empirical

evidence supporting this perspective has shown that reactive, but not proactive aggression is associated with an acute increase in physiological stress in response to a laboratory induced stressor, including higher skin conductance reactivity (Clanton, 2007; Hubbard et al., 2002) and higher salivary cortisol levels in 7-year-old children (see Clanton, 2007; Lopez-Duran, Olson, Hajal, Felt, & Vazquez, 2009). These findings suggest that proactive and reactive aggression may not only stem from different social information processing frameworks (Crick & Dodge, 1994, 1996), but also differ in their neurobiological stress mechanisms. What is missing from the literature are investigations examining the association between daily patterns of cortisol (i.e., not stress reactivity) and proactive and reactive subtypes of aggression. This is an important distinction given that investigations designed to index daily HPA activity through daily patterns in salivary cortisol are likely assessing the child's ability to regulate stress in a typical, daily environment. Whereas cortisol levels in response to an acute stressor are indexing HPA axis stress reactivity, or the child's ability to mount a stress response and the concomitant behaviours associated with the biological stress response. Thus, acute HPA axis stress reactivity may be more strongly associated with an outburst of reactive aggression compared to proactive aggression under conditions of acute stress (e.g., peer provocation). Hence, the associations of daily HPA activity to proactive and reactive aggression likely differ from those obtained under conditions of acute HPA axis stress reactivity. It is possible that in everyday, resting conditions, daily activity of the HPA axis is associated with both reactive and proactive aggression, whereas under conditions of acute stress, only reactive aggression is associated with HPA axis reactivity.

Of the limited research that has attempted to examine daily HPA axis activity in relation to proactive and reactive subtypes of aggression, no significant association has been observed in a sample of 7-year-old, healthy children (Lopez-Duran et al., 2009). However, this study was limited by sampling of 'basal, resting' cortisol in a laboratory context prior to a stress induced protocol. The authors acknowledged that the novel laboratory environment may have obscured

the ability to obtain a true baseline or basal measure of HPA axis activity. Only one other known published study to date has been designed to examine basal or daily HPA axis activity in relation to proactive and reactive subtypes of aggression (see Poustka et al., 2010). The findings from this study revealed an inverse association between afternoon plasma (blood) cortisol levels (5pm to 6pm) and parent-reported proactive and reactive aggression in a large sample of high-risk adolescents males. These results are consistent with the proposal that daily HPA axis activity is associated with both proactive and reactive aggression in the absence of an acute stressor. However, the results of this study were limited in three important ways. First, the results are restricted to plasma samples obtained by venipuncture within a laboratory setting, suggesting that the samples obtained were not representative of typical resting concentrations within naturalistic environments. A second important limitation was that only one cortisol sample was obtained. As mentioned previously, multiple samples of basal cortisol across the day are recommended to adequately index daily HPA axis activity (e.g., Adam, 2006; Cicchetti, et al., 2010; Murray-Close et al., 2008). Finally, the lack of statistical analyses beyond basic correlational comparisons limits the generalizability of the results towards understanding neurobiological predictors of children's behaviour. These limitations emphasize the need for methodologically sound investigations designed to examine the associations between daily cortisol patterns and proactive and reactive subtypes of aggressive behaviours in everyday contexts, such as classrooms.

***Social aggression.*** Limited research has explored the association between daily patterns of cortisol and social aggression in middle childhood. Based on the theoretical perspectives explicating how low cortisol is related to physical aggression or externalizing behaviours, there is reason to expect that a similar association may exist with social aggression. In a recent study of social aggression, Murray-Close and colleagues (2008) proposed that children who are chronically under-aroused (i.e., low daily cortisol or daily HPA axis activity) seek to engage in

socially aggressive behaviours such as gossip and rumour spreading with the goal of increasing their physiological arousal to more comfortable levels. Borrowing from theories of fearlessness, the authors propose that children may engage in social aggression as they have less fear of reprisal from their peers. To date, there remains a notable dearth of investigations exploring the associations between children's HPA axis activity and social aggression. Preliminary evidence suggests that lower levels of cortisol following the (9am) morning arrival at day camp and a more gradual decline in cortisol over the day are associated with higher ratings of peer-reported social aggression (Murray-Close et al., 2008). However, these results are limited to the context of a week-long summer day camp where the environment, counsellors, and peers were novel. It is possible that the peer- and counsellor-reported behavioural measures were not sufficient to capture typical, behavioural interactions characteristic of individual children. Social aggression is inherently a peer-based form of aggression, with rates shown to increase in middle childhood as children spend more time with peers and close friends (Crick et al., 1999). In contrast to summer day camps, classrooms offer a rich context in which to examine children's social aggressive behaviours, as peers spend many hours together across the school year. Teachers and peers each offer a unique perspective on which individual children are rated as more socially aggressive than others. Thus, an important avenue worth pursuing is the association of daily patterns of cortisol to peer- and teacher-reported social aggression in typical, everyday classroom contexts. Investigations that incorporate peer- and teacher- reports of social aggression may offer better insight into the neurobiological correlates of this peer-based form of aggression.

**HPA axis activity and prosocial behaviour.** Traditionally, investigations of behavioural development in middle childhood have focused primarily on the maladaptive consequences of HPA axis activation. However, the stress response system also plays a significant adaptive role in positive development, permitting children to respond to changes in their environment (e.g., DeVries, Craft, Glasper, Alexander, & Neigh, 2007; DeVries, Glasper, & Detillion, 2003;

Romeo & McEwen, 2006). Research shows that children high in prosocial behaviours are also high in constructive social skills, such as socially appropriate behaviour, coping, attentional-regulation, and low in negative emotionality (Eisenberg et al., 1996) suggesting that prosocial children may have better regulation of their stress response system. Surprisingly, minimal research has examined the role of the HPA axis in relation to prosocial, sharing, and helping behaviours during middle childhood. A theoretical model linking daily HPA axis activity to peer- and teacher- supportive relationships and prosocial behaviour in middle childhood will be proposed later in this chapter.

### **Summary of Daily Patterns of Salivary Cortisol and Behaviour in Middle Childhood (path c).**

The burgeoning number of investigations examining daily patterns in cortisol and aggressive behaviours in children in recent years is likely due to the ease of sampling cortisol in children's saliva. However, the value of these investigations is limited by a number of methodological inconsistencies. Limited research has explored what aspects of the cortisol diurnal rhythm (i.e., morning, afternoon, or slope) are more strongly related to specific subtypes of aggressive behaviour and prosocial behaviours in middle childhood. Research studies designed to address these limitations may permit more reliable interpretation of the association between daily patterns in salivary cortisol and prosocial and aggressive behaviours in everyday classroom contexts. Additionally, well-designed investigations will provide an opportunity to explore more advanced, integrative models of child development that take into account peer- and teacher- supportive relationships as a possible explanatory process by which cortisol influences social behaviour.

The following section introduces literature supporting an association of children's relationships with peers and teachers to children's social behaviour. Next, evidence is presented

suggesting that daily HPA axis activity in middle childhood is associated with behaviour via supportive relationships with peers and teachers. The ultimate goal of this review is to integrate the literatures towards a model where cortisol and supportive relationships with peers and teachers are better predictors of children's social behaviour than either variable alone.

### **Peer and Teacher Supportive Relationships and Children's Social Behaviour (path *b*)**

During middle childhood, approximately 30% of children's social interactions are with peers, compared to 10% in early childhood (Hamre & Pianta, 2001). Peers are powerful socialization agents, contributing beyond the family context to influence children's social, emotional, and academic adjustment (Harris, 1998; Rubin, Bukowski, & Parker, 2006). In addition, children start to develop relationships with non-familial adults such as teachers, who can become valuable sources of support (Hamre & Pianta, 2001; Jerome, Hamre, & Pianta, 2009; Myers & Pianta, 2008). Accordingly, children's relationships with peers and teachers start to play a more significant role during the middle childhood years (Levitt, Levitt, Bustos, Crooks, & Santos et al., 2005; Murray & Greenberg, 2000). The relationships that children develop with their peers and teachers can have immediate and enduring effects on students' motivation and social behaviour (e.g., Juvonen & Wentzel, 1996; Wentzel et al., 2004). Supportive relationships with peers and teachers provide children with a sense of security and support that promotes school engagement, prosocial behaviours (Wentzel, 2005; Wentzel et al., 2007), and subsequent academic, social, and emotional adaptation (Hamre & Pianta, 2001; Kasen, Johnson, & Cohen, 1990; LaRusso, Romer, & Selman, 2008; Myers & Pianta, 2008). In contrast, when children feel alienated or rejected in classroom environments, they are at risk of developing antisocial behaviours and poor academic achievement (see Buhs, Ladd, & Herald, 2006; Ladd & Burgess, 2001; Murray & Greenberg, 2000). Two broad areas of developmental theory guide the research on how children's peer and teacher supportive relationships influence their behavioural

development: the first is attachment theory (Bowlby, 1969; Ainsworth, Blehar, Waters, & Wall, 1978), and the second is self-determination theory (Connell & Wellborn, 1991; Ryan & Deci, 2000; Skinner & Belmont, 1993).

Attachment is a deep and enduring bond that connects a child to another individual across time and space (Ainsworth et al., 1978; Bowlby, 1969). Specific attachment behaviours in early childhood include showing preference for, or retreating to an attachment figure when threatened or upset, and using the attachment figure as a secure base to explore their environment. Research shows that young children with a history of maltreatment are at increased risk for disorganized attachment, depicted by conflicting patterns of avoidance and approach behaviour to a primary caregiver. Children who are unable to effectively use their caregiver as a resource to cope with challenge may be particularly vulnerable to changes in their developing stress response systems including dysregulation of the HPA axis (Tarullo & Gunnar, 2006, p. 633) and externalizing behavioural problems (see Fearon, Bakermans-Kranenburg, van IJzendoorn, Lapsley, & Roisman, 2010 for a meta-analytic study of the significance of insecure attachment in the development of children's externalizing behaviour). By middle childhood, children are typically attached to family members including the mother, father, and siblings, and may also be attached to non-family members such as peers and teachers (Bergin & Bergin, 2009). Attachment has two primary functions relevant to classrooms: it provides feelings of security so that children can explore freely, and attachment with others forms the basis for socialization with peers and teachers (Bergin & Bergin, 2009). Models of socialization stress the importance of children adopting parental and societal goals as a critical step towards their socialization into adult roles (Grusec & Goodnow, 1994; Juvonen & Wentzel, 1996, p. 233). The increased importance of peers in middle childhood is an indication of normal identity development, suggesting that children have managed to form attachments and obtain support outside of their primary family social network (Levitt, Guacci-Franco, & Levitt, 1993).

According to the motivational theory of self-system processes, also known as self-determination theory (SDT), children have three basic psychological needs: autonomy, belonging, and competence (Connell & Wellborn, 1991; Ryan & Deci, 2000) all of which can be met in a classroom through children's interactions with peers, teachers, and the learning environment. Self-determination theory (Ryan & Deci, 2000) further suggests that children whose basic needs of autonomy, belonging, and competence are met, develop better self-regulation skills (e.g., the ability to persist at difficult tasks or wait for one's turn) and exhibit greater social competence (Spinrad et al., 2006). The focus of this review is the theory of belonging, which suggests humans have a "persuasive drive to form and maintain at least a minimum of lasting, positive and significant interpersonal relationships" (Baumeister & Leary, 1995, p. 497). A sense of belonging is similar to attachment in that it can make children feel secure and valued which can liberate them to pursue academic and social challenges (Bergin & Bergin, 2009). Also referred to as a sense of relatedness (Connell, 1990) or connectedness (Weiner, 1990), a sense of belonging includes views about the self as lovable or unworthy of love and about the social world as trustworthy or hostile. Children rely on these beliefs when predicting, interpreting and responding to social exchanges (Furrer & Skinner, 2003). Social support with peers and teachers may function as a resource during middle childhood when children are faced with challenges or difficulties, permitting them to respond with more vigour, flexibility, and constructive and prosocial actions (Furrer & Skinner, 2003).

**The shifting role of peer and teacher support during middle childhood.** Evidence from the developmental literature highlights the importance of examining the relative importance and independent roles of peers and teachers in children's lives during middle childhood. Children's peer and teacher relationships become more important as children transition from childhood into adolescence (Darling, Hamilton, & Shaver, 2003; Levitt et al., 1993), however the literature suggests that teachers are important to children and young adolescents for different

reasons than are either peers or parents (Darling et al., 2003). Children are able to form relations with teachers that are qualitatively different from those relations with parents (van IJzendoorn, Sagi & Lambermon, 1992). Teachers represent a middle ground between parents and peers; they represent adult norms but are less charged with discipline compared to parents, which may foster greater communication (Darling et al., 2003). Evidence suggests that having a balance of peer and adult support has both an additive and positive influence on children's behavioural development (e.g., Buchanan & Bowen, 2008; Laible, Carlo, & Raffaelli, 2000). For example, students' perceptions of academic autonomy and both teacher- and peer-support are shown to have independent, positive effects on engagement in learning, and adjustment (Van Ryzin, Gravely, & Roseth, 2009). Therefore, research aimed at exploring the social-relational correlates of children's social behaviour needs to take into account the shifting role of peers and teachers during middle childhood and examine both the individual and collective contributions of peer and teacher support on children's behavioural outcomes.

**Peer supportive relationships and children's social behaviour.** According to a peer socialization perspective, peer relationships are thought to provide unique opportunities for children to learn and practice prosocial skills in a somewhat egalitarian and reciprocal fashion (Piaget, 1965; Wentzel & McNamara, 1999). Theorists propose that prosocial behaviours such as sharing and cooperation can only develop out of peer interactions that adopt principals of reciprocity and mutual respect (Hartup, 1992; Kohlberg & Kramer, 1969). Hence, children who are well-accepted by peers are more likely to display prosocial behaviours than their less-accepted counterparts (Criss, Shaw, Moilanen, Hitchings, & Ingoldsby, 2009; Wentzel & Erdley, 1993; Wentzel & Caldwell, 1997). Longitudinal research in kindergarteners supports the perspective that early peer acceptance (e.g., "classmates you would like to be in school activities with") provides children with a sense of belonging and inclusion in peer activities that decreases the propensity to disengage from academic activities and display aggressive behaviours (Ladd &

Burgess, 2001). Peer acceptance is shown to be pivotal in promoting positive adaptation during times of transition, such as school entrance (Ladd & Burgess, 2001). In support of a peer socialization perspective, Wentzel and McNamara (1999) found that peer acceptance, but not perceived support from peers or family, was directly and independently related to peer-nominated prosocial behaviour in middle childhood. In the long-term, peer acceptance and friendships in 6<sup>th</sup> grade are shown to predict prosocial behaviours and decrease emotional distress in 8<sup>th</sup> grade (Wentzel et al., 2004). Indeed a large corpus of evidence exists supporting significant associations of peer acceptance to friendships as well as social-emotional, and behavioural outcomes in middle childhood (see Asher, Parkhurst, Hymel, & Williams, 1990; Bierman, 2004; Criss et al., 2009). Evidence from a recent randomized controlled trial in young children followed from kindergarten to second grade, found that an intervention designed to promote positive peer relations, resulted in greater ratings of peer acceptance that mediated a decrease in teacher-rated externalizing behaviours (Witvliet, van Lier, Cuijpers, & Koot, 2009). Taken together, it would appear that peer acceptance is a significant component of positive behavioural development in middle childhood.

Peer rejection (e.g., “classmates you would not like to be in school activities with”), in contrast, is thought to increase a child’s dislike toward school, increasing classroom disobedience and decreasing classroom participation, which ultimately restricts children’s access and ability to participate in classroom activities (Ladd & Burgess, 2001; Ladd, Herald-Brown, & Reiser, 2008). Longitudinal research suggests that long-term, chronic peer rejection is more of a risk factor for maladjustment than early onset peer rejection (Ladd & Burgess, 2001). Indeed, evidence demonstrates that behaviour problems in the early school years are associated with peer rejection by middle childhood. Peer rejection in turn leads to fewer friendships and higher rates of adolescent internalizing disorders (Pedersen, Vitaro, Barker, & Borge, 2007). Peer rejection during the critical developmental period of middle childhood may impact the ability of children

to form positive peer relationships in the years to come. Indeed, peer rejected children are considered to be socially maladjusted and are at risk of poor school adjustment (Asher et al., 1990; Buhs et al., 2006; Ladd, 2006) as well as depression (Nesdale & Lambert, 2007; Oldehinkel, Rosmalen, Veenstra, Dijkstra, & Ormel, 2007; Schwartz, Gorman, Duong, & Nakamoto, 2008). In the long term, peer rejection is associated with psychological maladjustment (Lev-Wiesel, Nuttman-Shwartz, & Sternberg, 2006; Parker & Asher, 1987; Prinstein & Aikins, 2004) and later externalizing behaviours (Berdan, Keane, & Calkins, 2008; Ladd & Burgess, 2001; Laird, Jordan, Dodge, Pettit, & Bates, 2001). Notably, several longitudinal studies have shown that peer rejection mediates the association between early problem behaviour and later antisocial behaviour (Ladd & Troop-Gordon, 2003; Snyder, Prichard, Schrepferman, Patrick, & Stoolmiller, 2004; Vitaro, Pedersen, & Brendgen, 2007). These studies indicate that peer acceptance and peer rejection are not just markers of behaviours, but are necessary components for the development of children's prosocial and aggressive behaviours (Witvliet et al., 2009).

**Teacher supportive relationships and children's social behaviour.** Teacher support is thought to contribute to respectful school climates through increased feelings of social belonging (LaRusso et al., 2008). Teachers, through the quality of interactions with children and choice of classroom practices have the ability to create an environment that promotes or constrains children's psychological needs, which is reflected in children's behavioural and academic outcomes (Brock et al., 2008). Student-teacher closeness, characterized by warmth and open communication between student and teacher is thought to increase children's motivation to engage and participate in academic activities (Ladd & Burgess, 2001). Students who define teachers as uncaring and lacking in provision of autonomy and support are unmotivated to behave in prosocial ways, whereas those children who define teachers as emotionally supportive are motivated to be prosocial and socially responsible (Juvonen & Wentzel, 1996. p. 239).

Subsequently, children who report poor relationships with teachers and less connectedness to school have lower scores on self- and teacher-ratings of social and emotional adjustment and higher conduct problems compared to children who report positive teacher relationships and school connectedness (e.g., Birch & Ladd, 1998; Hamre & Pianta, 2001; Kuperminc, Leadbeater & Blatt, 2001; Loukas & Murphy, 2007; Murray & Greenberg, 2000; Pianta, 1992). Children with classroom behavioural problems tend to have decreased quality of relationships with teachers, which in turn promotes further behavioural difficulties- resulting in a vicious cycle (Birch & Ladd, 1998; Buyse, Verschueren, Doumen, Van Damme, & Maes, 2008; Hamre & Pianta, 2005; Sutherland & Oswald, 2005). Efforts to increase student-teacher closeness may buffer children at risk for maladaptive behaviours, through disruption of the cycle of behaviour problems and relational risk factors. Teachers' perceptions of the quality of relationships with their students can be assessed using the closeness subscale of the Student-Teacher Relationship Scale (STRS; Pianta, 2001; Pianta & Hamre, 2001). The closeness subscale is comprised of items that measure warmth and open communication in the teacher-student relationship (i.e., "I share an affectionate, warm relationship with this child"). In past studies, the STRS has been shown to be related to current and future academic and social functioning (Hamre & Pianta, 2001), behavioural adjustment, and peer relationships in pre-kindergarten through the elementary grades (Birch & Ladd, 1998; Jerome et al., 2009).

### **Summary of Peer and Teacher Supportive Relationships and Children's Social Behaviour**

Peer and teacher social interactions start to play a more influential role during middle childhood as children transition from the family nucleus towards more non-familial attachments. Positive social relations with peers and teachers in the classroom fulfill children's fundamental need to belong, promoting feelings of security, self-worth, and increased social motivation, resulting in long-term behavioural adaptation. Moreover, peer and teacher supportive

relationships represent a support system that facilitates children's ability to deal with the increased social challenges of middle childhood. In contrast, a lack of social support during times of stress may augment children's maladaptive behaviours and contribute to long-term physical and mental health problems. Research suggests that teachers are important to children for different reasons than peers, suggesting that the influences of supportive relationships with peers and teachers on children's behaviour are independent and unique. Missing from the literature is an examination of how children's neurobiological functioning is associated with peer acceptance and teacher closeness and subsequent behaviour. The following section will draw upon evidence in low-risk, typically developing children suggesting that dysregulation of HPA axis activity in middle childhood may influence children's ability to form supportive relationships with peers and teachers, which in turn influence their prosocial and aggressive behaviours.

### **Daily Patterns in Salivary Cortisol, Peer and Teacher Relationships, and Behaviour**

Classrooms represent an important social context in which to examine children's developing HPA axis in relation to social behaviours (Hamre et al., 2009). Surprisingly, a paucity of investigations have addressed the association between children's daily HPA axis activity and their supportive relationships with peers and teachers. Consistent with a chronic stress model, insecure attachment or low quality peer and teacher relationships stemming from early childhood is shown to increase the probability, magnitude, and range of adjustment and behavioural problems by middle childhood and adolescence (Buhs et al., 2006; Coie et al., 1992; Ladd, 2006). A possible neurobiological mechanism by which early care giving environments or social interactions may impact behaviour in middle childhood is through long-term alteration of children's developing HPA axis (Alink et al., 2008; Gunnar & Donzella, 2002). The following discussion will review evidence linking insecure attachment and chronic social stress (e.g.,

variations in maternal care giving and peer rejection) in early childhood to daily HPA axis activity in middle childhood. This line of reasoning provides the justification for the main hypothesis of this research project which is that children's daily HPA axis activity by middle childhood significantly influences children's ability to form supportive relationships with peers and teachers, which in turn influences behavioural development.

**Daily patterns in salivary cortisol and supportive relationships (path a).** Research in high- as well as low-risk, community samples of children suggests that variations in early social experience may impact young children's capacity to cope with stress leading to dysregulation of the HPA axis. Alterations in children's HPA axis activity in early childhood may place children at further risk for poor quality social relationships and subsequent behavioural problems by middle childhood. For example, in a recent review of studies involving children from adverse rearing environments, Gunnar and Quevedo (2007) presented evidence from a number of studies of low-risk samples suggesting that lower than expected morning cortisol may be related to variations in children's care giving within the normal or non-maltreating range. Parallel, yet independent evidence suggests that insecure attachment early in life is associated with dysregulation of HPA axis reactivity in young children (e.g., Gunnar, Brodersen, Nachmias, Buss, & Rigatuso, 1996), greater peer rejection, teacher-student conflict, and higher problem behaviours in preschoolers (Wood, Emmerson, & Cowan, 2004) and adolescents (Dykas, Ziv, & Cassidy, 2008). In preschoolers attending day care, teacher-student conflict is shown to be associated with increased cortisol levels (Lisonbee, Mize, Payne, & Granger, 2008). By middle childhood and early adolescence, empirical evidence from independent cross-sectional studies suggests that dysregulation of the HPA axis stress response system is associated with victimization by peers (e.g., "said mean things, called you names," Vaillancourt et al., 2008), peer rejection (Gunnar et al., 2003), social status in school-based hierarchies (West, Sweeting, Young, & Kelly, 2010), and social isolation (Sanchez-Martin et al., 2001; Sontag, Graber,

Brooks-Gunn, & Warren, 2008; Stroud et al., 2009; Turner-Cobb, Rixon, & Jessop, 2008). In contrast, when children experience supportive relationships or secure attachment, their neurobiological stress response system may be less taxed not only during stressful events, but also during rest. Indeed, evidence suggests that secure attachment appears to buffer young children's HPA axis reactivity to stress (Gunnar & Donzella, 2002) and decrease aggression and increase prosocial behaviour in adolescence (Dykas et al., 2008). However, minimal investigations have examined the associations of daily HPA axis activity to supportive relationships with peers and teachers and behaviour during middle childhood. The evidence reviewed above suggests that this is a rich avenue worth pursuing. The following section introduces a hypothetical model where children's supportive relationships with peers and teachers mediate the association between daily HPA axis activity and behaviour in middle childhood.

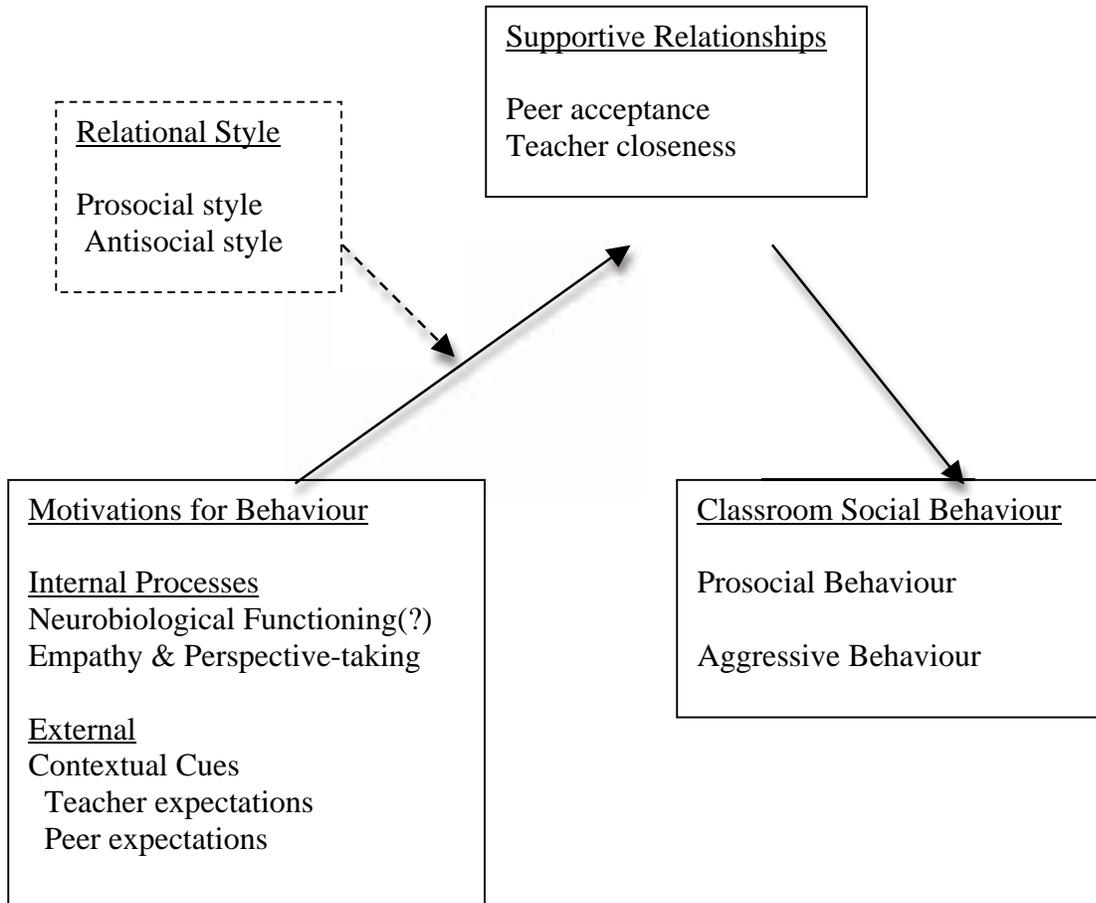
**The mediating influence of peer and teacher supportive relationships (path  $c'$ ).** A theoretical explanation of how daily patterns in salivary cortisol and peer and teacher supportive relationships influence social behaviour in middle childhood emerges from the work by Wentzel, Filisetti, and Looney (2007) on their conceptual model of adolescent prosocial behaviour, in conjunction with Ladd and colleagues' (1999) work on 'relational styles' that predict the types of relationships children form with peers and teachers. Based on developmental theories of classroom social motivation (Wentzel et al., 2004) and self-determination theory (Ryan & Deci, 2000), Wentzel and colleagues (2007) propose that children and adolescents have multiple external and internal reasons guiding their prosocial behaviour. External contextual cues such as children's perceptions of peers' and teachers' expectations for prosocial behaviour are shown to be significant motivations for behaving prosocially, whereas self-processes such as empathy and perspective-taking are thought to reflect internal motivations for prosocial behaviour. What is

lacking from this model, however, is a consideration of internal biological processes as motivational predictors of prosocial and aggressive behaviour in middle childhood.

Research by Ladd and colleagues (1999) on children's relational style offers insight into the association between internal biological arousal, peer and teacher interactions, and behaviour. For example, Ladd and colleagues suggest that relationship formation is based on the proposition that children seek to establish and maintain relationships in which they can maximize rewards such as arousal and common interests, while minimizing costs such as punishment and negative affective states (Ladd et al., 1999). The authors show that children with specific relational styles such as prosocial orientations are better able to form close relationships with peers and teachers. In contrast, children with antisocial relational styles are at risk for peer rejection and poorer quality student-teacher relationships. Thus, a child's relational style, or propensity to move towards or against others can either be a risk or a protective factor for prosocial and aggressive behaviours. Similar to the model proposed by Wentzel and colleagues (2007), what is not addressed in this model of relational style and behaviour is the motivation underlying children's relational style. Ladd and colleagues hint at 'arousal' as a motivation for social behaviour, but do not elaborate further. Expanding on this work, a plausible model is that children's daily HPA axis activity represents an internal biological process that underlies prosocial and antisocial relational styles, which in turn influences children's ability to form supportive relationships with peers and teachers. The quality of peer and teacher relationships subsequently influences children's prosocial or aggressive behavioural tendencies (see Figure 2). Current perspectives on neurobiological arousal and aggressive behaviour suggest that children with biological underarousal such as low HPA axis activity seek sensation through negative interactions with others. These negative interactions may constitute an antisocial relational style consequently leading to peer rejection, lower teacher closeness, and an increased propensity for aggression (e.g., Murray-Close et al., 2008; Raine, 2002). Expanding this theory to the developmental correlates of

prosocial behaviour, it is possible that specific aspects of internal biological arousal, undefined as yet, lend to a higher activation threshold in response to typical, everyday stimuli. Thus, children with a higher activation threshold may have better ability to cope with social interactions, which translates into a prosocial relational style that facilitates positive peer and teacher relationships and prosocial behaviours. Consistent with this perspective, emerging research is starting to explore the influence of social context on children's daily HPA axis activity and aggressive behaviours (e.g., Cicchetti et al., 2010; Murray-Close et al., 2008). Of the research examining social context and HPA axis activity in low-risk samples of children, the majority of studies have focused on preschool children in child care settings (e.g., Groeneveld, Vermeer, van IJzendoorn & Linting, 2010; see also Vermeer & van IJzendoorn, 2006; Watamura et al., 2009, in press). Evidence suggests that the quality of the care giving environment in home-based versus centre based day care settings influences daily patterns in salivary cortisol (e.g., Ouellet-Morin et al., 2010; Watamura et al., 2003, 2009). However, little research is available specific to classroom settings, a salient developmental context for older children in middle childhood. To address this gap, the current cross-sectional study was designed to explore the influence of peer acceptance and teacher closeness as an explanatory process by which daily patterns in salivary cortisol influence prosocial and aggressive behaviours in classroom settings in middle childhood. A series of multiple mediation analyses were performed to empirically examine the proposed hypothesis that peer acceptance and teacher closeness uniquely mediate the association between HPA axis activity and behaviour in middle childhood. This work was based on the hypothesis that HPA axis activity predicted, or preceded, the mediators- peer acceptance and teacher closeness, which in turn predicted children's behaviour. A caveat to a cross-sectional study of this nature however, is the lack of temporal precedence of variables, or the inability to adequately measure whether the predictor variable, HPA axis activity, preceded in time, the mediators and dependent variable, behaviour. As such, the alternative model is

Figure 2. Hypothetical model linking internal neurobiological functioning to prosocial and aggressive behaviour (adapted from Wentzel, Filisetti, & Looney, 2007).



possible, where children's behaviour (e.g., aggression) could precede poorer quality relationships with peers and teachers, which in turn may manifest as physiological stress and dysregulation of the HPA axis. In other words, behaviour could be the independent variable and biology (i.e., HPA axis activity) the dependent variable. To address this issue a series of exploratory analyses were performed to examine the mediating influence of peer and teacher relationships on the association between HPA axis activity and behaviour, with HPA axis as the dependent variable. The purpose of this exploratory work was to simply illustrate that analytical models that include relational-contextual variables offer more valuable information than examining biological correlates of behaviour alone.

### **Summary of Daily Patterns in Salivary Cortisol, Peer and Teacher Relationships, and Behaviour**

Evidence exists linking insecure attachment and chronic social stress (e.g., peer rejection) in early childhood to dysregulation of HPA axis activity in middle childhood. Independent lines of evidence suggests that children's daily HPA axis activity is a measure of biological arousal, and influences children's internal motivations to engage in social interactions and prosocial or aggressive behaviour. Children with dysregulation of daily HPA axis activity in middle childhood may exhibit a diminished capacity to cope with stress or challenge, placing them at risk for lower peer acceptance, lower teacher closeness and an increased propensity for aggression. This theoretical line of reasoning forms the basis of the proposed study hypothesis, where supportive relationships with peers and teachers mediate the association between children's daily HPA axis and behaviour in middle childhood. The following section introduces the conceptual basis of mediation analyses and reviews traditional as well as more sophisticated approaches to mediation analyses.

## **Mediation Model**

The prevailing view within developmental psychobiology and more integrative perspectives of child development, is that hormones and behaviour mutually influence each other and that this interaction is both mediated and moderated by environmental or contextual factors (Boyce et al., 2002; Gunnar & Quevedo, 2007; Susman & Ponirakis, 1997). Although the literature on behavioural development during middle childhood has advanced to the point where model development is now possible, several attempts to identify mediating factors that facilitate adjustment have not taken full advantage of the terminological, conceptual, or statistical advances that would facilitate progress in the field (Holmbeck 1997; Preacher & Hayes, 2008). The following discussion reviews the theory and concept underlying mediation analyses. Three common but conceptually distinct approaches to mediation analyses will be introduced to illustrate the inconsistencies and advantages inherent to different approaches.

**Mediation model: causal not correlational.** Mediation is a causal model that attempts to explain the intermediary process that leads from an independent variable, such as HPA axis activity to a dependent variable such as behaviour. Theoretically and conceptually, mediating mechanisms are proposed only if a body of literature has tentatively indicated a causal relationship between an independent variable and a dependent variable (Rose, Holmbeck, Coakley, & Franks, 2004). However evidence of absolute causal factors that “cause” specific behavioural outcomes in 100% of cases are rare in behavioural research. It is more likely that multiple causal factors cause children’s behaviours, as well as reciprocal associations where behaviour may influence predictors such as daily HPA axis activity (Bauman, Sallis, Dziewaltowski & Owen, 2002). As such the available literature cannot characterize the relationship between children’s HPA activity and behaviour as ‘causal’. Notwithstanding, the field is replete with studies illustrating statistically significant correlational associations between cortisol and behaviour. Such relationships do not support causal inferences, but may generate

hypotheses for further study. As such, it is appropriate to state upfront that the use of a mediation model in the present study was not to attempt to examine causal relationships per se, but rather to use mediation as a heuristic model if only to gain insight into the *processes* by which daily patterns in cortisol influence behaviour. The strengths and limitations of this mediation approach are addressed in the interpretation of the results and, as recommended by Wu and Zumbo (2008), the phrasing of the results remain correlational.

**What is mediation?** Mediation is not defined statistically; rather statistics are used to evaluate a proposed mediation model (see Wu & Zumbo, 2008). In the simple mediation model proposed in the current study, the independent variable, daily HPA axis activity is presumed to influence the mediators, peer acceptance and teacher closeness (supportive relationships), that in turn influence the dependent variable, social behaviour (Preacher & Hayes, 2008; Wu & Zumbo, 2008; see Figure 1). The influence of daily HPA axis activity on behaviour can be allocated into its direct effect on behaviour (path *c*) and its indirect effect on behaviour through the mediator (path *c'*). The direct effect is interpreted as the part of the effect of daily HPA axis activity on behaviour that is independent of the pathway through the mediator. The indirect effect is interpreted as the amount by which two cases who differ by one unit on daily HPA axis activity are expected to differ on the behavioural outcome through daily HPA axis activity's influence on supportive relationships, which in turn influences behaviour (Hayes, 2009, p. 409). In Figure 1B, path *a* represents the influence of daily HPA axis activity on the proposed mediator - supportive relationship - while path *b*, is the influence of supportive relationships on behaviour, controlling for the influence of daily HPA axis activity. The paths among these variables are typically quantified using unstandardized regression coefficients. The product of path *a* and path *b*, can then be calculated to give the indirect effect of daily HPA axis activity on behaviour through supportive relationships (i.e., *ab*). The total effect of daily HPA axis activity on behaviour, can then be expressed as the sum of the direct and indirect effect ( $c = c' + ab$ , see Preacher & Hayes,

2008, p. 879). Or, to state another way, the indirect effect ( $ab$ ), is the difference between the total and direct effect of daily HPA axis activity on behaviour ( $ab = c - c'$ ).

**Approaches to mediation analyses.** A number of different methods for testing hypotheses about mediation have been proposed (see MacKinnon, Lockwood, Hoffman, West, & Sheets, 2002 for a review). Indeed, the methodological literature is replete with discussions critiquing traditional approaches to mediation analyses while extolling novel methodological concepts and applications. To take advantage of these intersecting schools of thought, the current study will utilize three different mediation approaches to emphasize the strengths and limitations of each. First the traditional ‘causal steps’ approach made popular by Baron and Kenny (1986) will be introduced, followed by the ‘product of coefficient’ approach using the Sobel test (MacKinnon et al., 2002; Sobel, 1982, 1986). Finally, the benefits of the ‘bootstrapping of indirect effects’ approach (MacKinnon, Lockwood, & Williams, 2004) made popular by Preacher and Hayes (2008) will be presented and ultimately used to interpret the data. These three approaches have been discussed at length by Preacher and Hayes (2008; Hayes, 2009) in their recent article on assessing and comparing indirect effects in mediation models and are summarized below.

***Causal steps approach.*** The most commonly used approach is the causal steps method in which the investigator estimates the paths of the model in Figure 1B, using ordinary least squares regression, and assesses the extent to which several criteria are met (Baron & Kenny, 1986). The causal steps approach includes four steps to establish mediation: (1) the independent variable, daily HPA axis activity is correlated with the outcome, behaviour- path  $c$ ; (2) the independent variable, daily HPA axis activity significantly accounts for variability in the mediator- path  $a$ , (3) the mediator, significantly accounts for variability in the dependent variable, behaviour, when controlling for daily HPA axis activity - path  $b$ , and (4) the direct effect of daily HPA axis activity on behaviour - path  $c$  decreases substantially when the mediator is entered

simultaneously with daily HPA axis activity as a predictor of behaviour- path  $c'$  (Baron & Kenny, 1986). According to Baron and Kenny, if all four steps are met, then the data are consistent with the hypothesis that the mediator, supportive peer and teacher relationships, completely mediates the association between daily HPA axis activity and behaviour. If step 4 is not met, then partial mediation is indicated. This reasoning has received much criticism and, with the emergence of more theoretically sound models, it is suggested that the constructs of complete and partial mediation are now redundant (Preacher & Hayes, 2008; Hayes, 2009). Furthermore, debate exists in the literature regarding the need to fulfill all four steps of the causal steps approach. Step 4 does not need to be met unless complete mediation is expected – which is the exception rather than the norm. Step 1 is not required although it is implied if steps 2 and 3 are met (Kenny, Kashy & Bolger, 1998). Indeed, the causal steps approach has been criticized heavily on multiple grounds, most notably this method is shown to be among the lowest in power to detect a mediating effect (Fritz & MacKinnon, 2007; Preacher & Hayes, 2008; MacKinnon et al., 2002). The requirement for a significant direct effect between predictor and outcome before pursuing mediation models, may limit the behavioural researcher from finding a significant mediation effect. For example, it is entirely possible that the indirect effect through a mediator such as peer acceptance is a stronger predictor of the effect of daily HPA axis activity on behaviour, than the direct effect of daily HPA axis activity on behaviour alone. In other words, the presence of the third, mediating variable explains why no significant direct effect is observed. As such, investigations that report no significant direct effect between children's daily HPA axis activity and behaviour, are subject to 'left out variable error' – the lack of intermediary, explanatory variables. Irrespective of these limitations and the availability of more appropriate methods, the causal steps approach is still widely used – perhaps due to its simplicity and popularity. It is therefore informative to compare and contrast this

method with more advanced approaches as an illustration of the pitfalls and inconsistencies facing behavioural researchers interested in mediation analyses.

***Product of coefficient approach.*** Since the inception of the causal steps approach, additional approaches have been used to test mediation hypotheses that do not focus on the individual paths, but rather on the product term- path  $ab$ , or the indirect effect. Similar to the causal methods approach, multiple regression is employed to obtain the unstandardized regression coefficients for path  $a$  and path  $b$ . The Sobel test, also called the product of coefficients approach divides the mediated effect,  $ab$  by its estimated standard error (Sobel, 1982, 1986). To test mediation, a  $p$  value is obtained based on the standard normal distribution. Considering that the sampling distribution of  $ab$  is only normal in large samples, the product of coefficient approach has also received much criticism and bootstrapping approaches of the indirect effect are now becoming more popular in the developmental sciences.

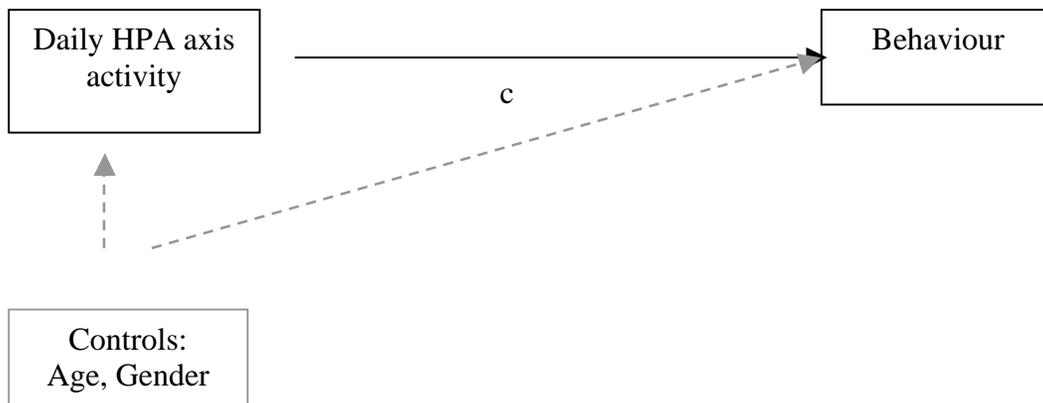
***Bootstrapping of simple indirect effects.*** Bootstrapping, a nonparametric re-sampling procedure is a more recent method for testing mediation that does not impose the assumptions of normality of the sampling distribution. Bootstrapping is a computationally intensive method that involves repeatedly sampling from the data set to estimate the indirect effect in each re-sampled data set. By repeating the process thousands of times, an empirical approximation of the sampling distribution of  $ab$  is obtained and used to estimate asymmetric confidence intervals for the indirect effect (MacKinnon, et al., 2002; Preacher & Hayes, 2008; Hayes, 2009). In other words, the individual paths are no longer pertinent to hypotheses about mediation, and inference is based on the product of  $a$  and  $b$  with bootstrap confidence intervals to test the indirect effect. Thus, there is no  $p$  value for the bootstrap method and sample size becomes less relevant (see Hayes, 2009). MacKinnon and colleagues (MacKinnon et al., 2004) examined the Type 1 error rates and power among the three methods; causal steps, product of coefficient and bootstrapping in extensive sets of simulations. They found that the bootstrapping method had greater power

while maintaining a reasonable Type I error rate, and as such it has been recommended that the bootstrapping method is used over the Sobel test or causal steps approach for testing hypotheses of indirect effects. Building on this approach, a design that is gaining more attention in both the methodological and applied literature involves simultaneous mediation by multiple mediators, i.e., multiple mediation (Preacher & Hayes, 2008). This multiple mediation model is more aptly suited to investigations such as the current study that are examining more than one putative mediator, i.e., peer acceptance and teacher closeness, in their model of child social behaviour.

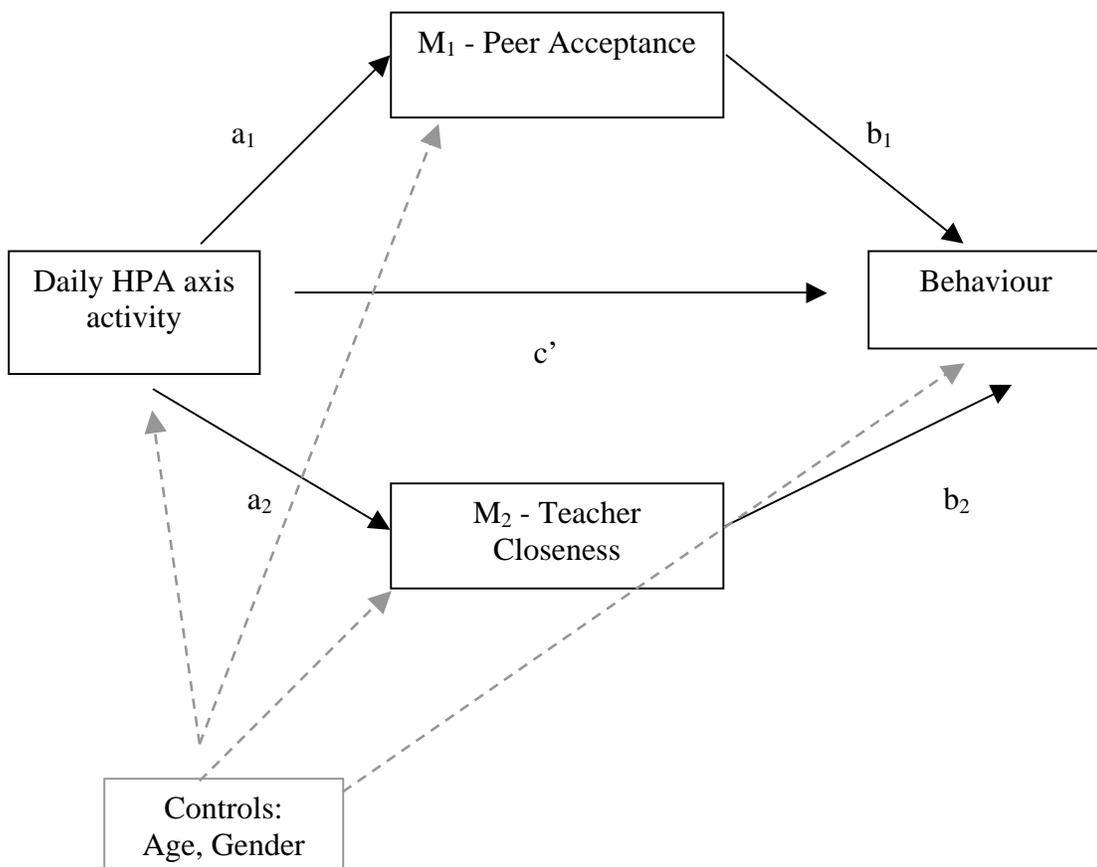
***Bootstrapping of multiple indirect effects.*** The method of multiple mediation was recently made popular by Preacher & Hayes (2008; Hayes, 2009) whose internet-based Facebook© group ‘statistical mediation analyses’ adequately captures the essence of their recent article, ‘statistical mediation analysis in the new millenium’ (Hayes, 2009). Multiple mediation involves the same logic as simple mediation (see Figure 3). For instance, in a proposed model with two mediators, the total effect is equal to the direct effect of daily HPA axis activity on behaviour plus the sum of the indirect effect through Mediator<sub>1</sub> (i.e.,  $a_1b_1$ ), plus the indirect through Mediator<sub>2</sub>, (i.e.,  $a_2b_2$ ). The indirect effect through a given mediator is called a specific indirect effect (also known as the point estimate). The sum of the specific indirect effects, i.e.,  $a_1b_1 + a_2b_2$  is called the total indirect effect of daily HPA axis activity on behaviour (see Hayes, 2009; Preacher & Hayes, 2008). However, the total indirect effect is rarely of interest in multiple mediation models, because when specific indirect effects are in opposing directions they can yield a total indirect effect that is insignificant even when the specific indirect effects are large. Thus, interpretation usually focuses on the ‘unique’ influence of the specific indirect effects of the mediators.

Figure 3. Multiple mediation model. (A) Illustration of a direct effect, where daily HPA axis activity influences behaviour, controlling for age and gender. (B) Illustration of a multiple mediation model. Daily HPA axis activity is hypothesized to exert indirect effects on behaviour through multiple mediators,  $M_1$  and  $M_2$ , controlling for age and gender (adapted from Preacher & Hayes, 2008).

**A**



**B**



Another important concept and advantage to the use of the multiple mediation approach is that a specific indirect effect in multiple mediation is not the same as the estimate of the indirect effect of a mediator alone. To be precise, it is the effect of one mediator, (e.g., peer acceptance) to mediate the influence of daily HPA axis activity on behaviour, conditional on the inclusion of another mediator (e.g., teacher closeness) and covariates such as age and gender. Thus, the multiple mediation model is suitably designed to empirically test the hypothesis that peer and teacher supportive relationships play a distinct or unique mediating role on the association between daily HPA axis activity and behaviour in middle childhood. Although the use of multiple mediation models are relatively novel to the behavioural literature, there are numerous advantages to including more than one putative mediator in the same model (see Preacher & Hayes, 2008, p. 881). To summarize, the benefits of a multiple mediation model over simple mediation models include the ability to: (1) examine both proposed mediators simultaneously to obtain indirect effects; (2) estimate how one mediator influences the association between daily HPA axis activity and behaviour, controlling for the presence of the other mediator; (3) reduce measurement bias due to omitted variables, and (4) determine relative magnitudes of each mediator with the other, a process that permits theory comparison – which, according to Preacher & Hayes, (2008, p. 881), is “good scientific practice”.

### **Summary of Mediation Model**

Recent advances in the field of mediation analyses permit the modelling of multiple mediators to examine the unique influence of one putative mediator, controlling for the presence of the other mediator. This model is ideally suited to theoretical models of child development such as that proposed in the current study, where the mediating influence of peer acceptance is thought to be distinct from the mediating influence of teacher closeness on children’s social behaviour. However, traditional methods of mediation analyses still remain popular in the

developmental literature despite significant limitations. As such, this study will employ three different approaches to mediation analyses to emphasize the strengths and limitations of each. This research will ultimately employ the approach of bootstrapping of multiple indirect effects to explore the unique mediating influence of peer and teacher supportive relationships on the association between daily salivary cortisol and classroom behaviour.

### **Summary of the Research**

Children's prosocial and aggressive behaviours during middle childhood are significantly associated with short- and long-term social, emotional adjustment and academic success (e.g., Brook & Newcomb, 1995; Caprara et al., 2001; Coie et al., 1992; Huston, & Ripke, 2006; Kokko et al., 2006). Therefore, research devoted to examining the internal, biological and external, relational correlates of children's behaviour, and how these factors interact to facilitate positive behavioural development is of great importance (Bronfenbrenner, 2005; Hamre et al., 2009; Pianta et al., 2008; Rutter, 1990; Wentzel, et al., 2007). As such, the present study was designed to expand our current understanding of behavioural development by examining the mediating influence of peer and teacher supportive relationships on the association between daily patterns in salivary cortisol and prosocial and antisocial behaviours in middle childhood. This study was based on the theoretical foundation that early experience can lead to dysregulation of the HPA axis in middle childhood that subsequently influences children's behaviour (Cicchetti et al., 2010; Gunnar & Quevedo, 2007). Cross-sectional research using salivary measures of cortisol in school-age children support this theoretical model, suggesting that children's HPA axis activity is significantly associated with behavioural development in middle childhood (e.g., El Sheik et al., 2008; Murray-Close et al., 2008).

The literature examining the association between daily patterns in salivary cortisol and children's social behaviours reveals three central themes that contribute to a more integrative

model to study biological correlates of behaviour in middle childhood. First, the majority of investigations examining cortisol and aggression are limited by a number of methodological inconsistencies (Jessop & Turner-Cobb et al., 2008). Most notable is the lack of specific indicators of daily patterns of salivary cortisol in children in everyday, naturalistic contexts such as classrooms. Second, the majority of studies examining daily patterns in salivary cortisol in relation to behaviour have failed to account for subtypes of aggression that are shown to be differentially related to specific indices of psychosocial adjustment (Little et al., 2003; Murray-Close & Ostrov, 2009). Limited research has examined the association between salivary cortisol and prosocial behaviours in middle childhood. Third, theoretical and empirical evidence exists supporting the unique influence of peer acceptance and teacher closeness as mediating, explanatory variables in the association between daily patterns in salivary cortisol and behaviour in middle childhood (Ladd et al., 1999; Ouellet-Morin et al., 2010; Watamura et al., 2003, 2009; Wentzel et al., 2007). The additive combination of peer and teacher supportive relationships may be optimal for behavioural development in middle childhood (Buchanan & Bowen, 2008). Examination of how peer and teacher supportive relationships and daily patterns in cortisol are interwoven to account for children's social behaviour is a more productive theoretical and empirical strategy than viewing these variables as alternative accounts of children's social behaviour. Research aimed at identifying the neurobiological and social-relational correlates of specific subtypes of aggressive and prosocial behaviours in middle childhood have the potential to inform future classroom-based research efforts designed to promote positive behavioural development (see Beauchaine et al., 2008; Cicchetti & Gunnar, 2008; Hamre & Pianta, in press).

### **Purpose**

This study was designed to examine, (1) the associations of daily patterns of salivary cortisol to peer- and teacher-rated; reactive, proactive, and social aggression and prosocial classroom behaviours, and (2) to investigate the unique mediating influence of peer acceptance

and teacher closeness on the association between daily patterns of salivary cortisol and behaviour in a typical classroom context in middle childhood.

This cross-sectional study took place in four elementary classrooms in grades 4 and 5 during the Spring semester. Thus, measures of children's social behaviours, and peer and teacher supportive relationships were representative of the classroom in which children and teachers had been part of for the majority of the school year. Samples of children's salivary cortisol were obtained three times a day across four days in a classroom setting to provide a variety of indicators of daily patterns of salivary cortisol (i.e., average morning, noon and afternoon levels, area under the curve cortisol, and diurnal slope). Previous research suggests that cortisol concentrations at specific times in the day (i.e., morning, afternoon) and, flatter or steeper change in cortisol across the day (i.e., diurnal slope) are associated with specific subtypes of aggressive behaviours. Therefore, the association of various indicators of daily patterns in salivary cortisol to specific subtypes of aggressive behaviour were explored. Based on prior research suggesting that HPA axis dysregulation (i.e., flatter slope, or higher morning and lower afternoon levels) is associated with reactive, proactive and socially aggressive behaviours in middle childhood (Murray-Close et al., 2008; Poustka et al., 2010), it was predicted that low cortisol, indicative of biological under-arousal, would be associated with higher ratings of reactive, proactive, and social aggression. Associations between daily patterns of cortisol and prosocial behaviour were exploratory since no previously published studies have examined cortisol in relation to prosocial behaviour in middle childhood. Substantial theoretical and empirical evidence suggests that children's level of peer acceptance and teacher closeness uniquely mediate the association between daily patterns of cortisol and behaviour. This hypothesis was tested using traditional and more sophisticated approaches to mediation analyses at the level of the individual child. To control for potential effects of classroom environment at the classroom level, all multiple mediation models controlled for children's classroom. Due to

the cross-sectional nature of the design, the focus of this research was not to explore the influence of developmental effects or children's age. However, based on the empirical evidence that children's age, as well as gender, potentially influence the neurobiological, behavioural, and relational variables- all regression analyses controlled for age and sex. Given the cross-sectional nature of the study and lack of temporal precedence of variables for mediation analyses; the alternative model, with behaviour as the independent variable and HPA axis as the dependent variable, was addressed. This exploratory analyses serves only to illustrate the importance of including social-contextual variables, such as supportive relationships with peers and teachers, as mediating variables in biological studies of behavioural development.

### **Hypotheses**

In order to meet the purposes of this study, a number of hypotheses and exploratory research questions were put forth:

1. Dysregulation of daily HPA axis activity, indexed by daily patterns of salivary cortisol obtained in a classroom setting will be significantly associated with peer- and teacher-reported; 1) reactive aggression, 2) proactive aggression, and (3) social aggression.
2. Is there an association between daily patterns of salivary cortisol and prosocial behaviours?
3. Peer acceptance and teacher closeness will uniquely mediate the association between daily patterns of salivary cortisol and children's behaviours.

## CHAPTER 3

### Method

#### Participants

Participants were 89 children (49 girls, 40 boys) drawn from four regular education classrooms in the fourth and fifth grades of a public elementary school located in a large Western Canadian city ( $M_{\text{age}} = 10.44$  years,  $SD = .63$ ,  $Range = 9.26 - 12.18$ ). This research was part of a larger study designed to examine biological, social, and emotional correlates of child development in school-age children residing in classroom contexts. Consent was obtained from the university ethics board and school district and written informed parental consent and child assent were obtained prior to children's participation in the study. Of the 106 children that were invited to participate in the study from all four classrooms, 84% of them received parent/guardian permission to participate. Children who were identified by teachers as not competent in written and spoken English (1%;  $n = 1$ ) were excluded from participation. The school in which children were recruited included a diverse range of socioeconomic status and was considered to be a microcosm of the larger community, containing families with service workers, skilled labourers, and professionals. Classroom teachers were all female (3 Caucasian, 1 Asian). As to participating children's family composition, 62 % ( $n = 55$ ) of children reported living in two parent homes (including both biological and step-parent families), 25 % ( $n = 22$ ) reported living with a mother only, 1% ( $n = 1$ ) father only, 1% ( $n = 1$ ) grandmother only and the remainder, 11% ( $n = 10$ ) reported living in dual custody arrangements (i.e.,  $\frac{1}{2}$  time mother,  $\frac{1}{2}$  father). The majority of children reported English (67%) as their first language followed by Chinese (15%). The remaining children (18%) indicated a range of other language backgrounds (e.g., Spanish, Vietnamese, Tagalog) reflective of the cultural and ethnic diversity of the Canadian city in which this study took place.

## **Procedure**

Following approval by the University of British Columbia Behavioural Research Ethics Board (BREB) and Vancouver School Board (see Appendix A), and prior to providing the children with the parental permission slips, either a trained research assistant or the Principal Investigator of the research project provided a 15-minute presentation to each participating class describing the study in age appropriate language and answering children's questions. Upon receiving parental consent and child assent research assistants administered a series of questionnaires to the children during a regular 40-minute class period (see Appendix B). The instructions for each questionnaire and the questionnaire items were read aloud. Students were informed they did not have to answer any of the questions if they did not want to. Within two weeks, two research assistants visited each individual classroom three times a day over four consecutive days (i.e., Tuesday through Friday) to collect saliva samples. Salivary cortisol samples were obtained from each child at the same time of day; morning, noon, and afternoon (i.e. 9am, 12pm, and 3pm). To ensure that teachers would have adequate time to complete the behaviour rating scales for each of their students, each teacher was provided with a half day Teacher on Call. Students and teachers were informed that their responses would be confidential and that data would be stored in a secure facility that was accessible only to the research team. All parents and teachers signed consent forms and students signed assent forms that stated assurances of confidentiality (see Appendix C). Data were gathered in the Spring semester of the school year so that teachers and students had adequate time to know one another. The sample consisted of 89 students. One student was not present at time of administration of the questionnaires, but was available to complete all questionnaires at a later date, except for peer-nominated behavioural ratings.

## Measures

The questionnaire package administered to the children in this study contained previously validated instruments assessing peer-nominated social behaviours and peer acceptance along with a series of questions on students' demographic information. Teacher ratings were used to assess student-teacher closeness as well as student prosocial and aggressive behaviours. For all questions, teachers and students were instructed to answer with reference to their experiences in the class they were in at the time of data collection (see Appendix B).

**Demographic information.** Students completed a general information sheet that asked their age, gender, family composition, date of birth, first language learned, history of illness, and current medications (see Appendix B-1).

**Teacher-reported social behaviours.** Teachers rated students' prosocial behaviour and social aggression (National Longitudinal Survey of Children and Youth, 2001; Statistics Canada and Human Resources Development Canada, 2001) and proactive and reactive aggression (Crick & Dodge, 1996; Dodge & Coie, 1987; see Appendix B-2). For each student, teachers rated 27 items on their feelings and beliefs about the student's behaviours on four subscales; prosocial behaviour (10 items, e.g., "will try to help someone who has been hurt;"  $\alpha = .94$ ); proactive aggression (9 items, e.g., "threatens or bullies other children to get his/her own way," "plays mean tricks;"  $\alpha = .84$ ); reactive aggression (3 items, "e.g., when teased or threatened, he/she gets angry easily and strikes back;"  $\alpha = .86$ ); and social aggression (5 items, e.g., "when mad at someone, tries to get others to dislike that person;"  $\alpha = .85$ ). Teachers rated how applicable each statement was to the behaviour of the particular student on a 3-point scale: Never or not true (1), Sometimes or somewhat true (2), Often or very true (3). Items were averaged to yield a composite score with higher scores representing greater levels of prosocial and aggressive behaviours.

The subscales of prosocial behaviour and social aggression were developed with school-age children as part of a Canadian National Longitudinal Survey of Children and Youth (NLSCY, 2001). The subscales of proactive and reactive aggression were developed with 8 to 11 year-old children (Dodge & Coie, 1987). The reliability coefficients obtained in the present study are comparable to those obtained in previous studies with school-age children (Broidy et al., 2003; Crick & Dodge, 1996; Lee, Baillargeon, Vermunt, Wu, & Tremblay, 2007). The subscales are shown to have good predictive value for aggressive behaviours (Broidy et al., 2003; Card & Little, 2006; Card et al., 2008; Cote, Vaillancourt, LeBlanc, Nagin, & Tremblay, 2006; Vaillancourt et al., 2007) and prosocial behaviours in middle childhood (Pagani et al., 2006; Romano, Tremblay, Boulerice, & Swisher, 2005).

**Peer nominations of social behaviour.** Following the procedure of Parkhurst and Asher (1992) and Schonert-Reichl (1999), peer nominations on 13 questions were used to obtain independent assessments of social behaviour (see Appendix B-3). Below each written question, students were given a list of all classmates who were participating in the study. For each question, students were asked to circle the names of any of their classmates who fit the behavioural description. Students could circle as many or as few names as they wanted as well as their own name. Data collection took place at the end of the Spring semester when students had been with the same teacher and classmates for the school year, therefore it is reasonable to assume that the students knew each other well. For each question, the percentage of nominations each student received was computed by dividing the number of nominations received by the total number of participating students in the classroom. Composite scores were computed by averaging the nominations given by boys and girls. The distribution of students' composite scores were normalized using an arcsine square-root transformation (e.g., Oberle, Schonert-Reichl, & Thomson, 2009; Schonert-Reichl, 1999; Wentzel & Erdley, 1993). Four types of

social behaviours were assessed: prosocial behaviour (e.g., “shares and cooperates,” and “helps other kids when they have a problem;”  $\alpha = .89$ ; Wentzel & Erdley, 1993); reactive aggression (e.g., “gets angry easily and fights back when teased,” and “gets mad at kids who hurt them by accident;”  $\alpha = .89$ ); proactive aggression (e.g., “plays mean tricks or plans to hurt others,” and “gets other students to gang up on a classmate;”  $\alpha = .90$ ); and social aggression (e.g., “talks behind other people’s back,” and “when mad at someone, says, “lets not be his/her friend;”  $\alpha = .70$ ). These behaviours are shown to correspond to positive and negative interactional qualities that correlate with peer liking and disliking among children and young adolescents (see Oberle et al., 2009; Schonert-Reichl, 1999; Parkhurst & Asher, 1992; Oberlander et al., 2006). Higher scores represent greater levels of the behaviour. It has been established that this measure yields reliable and valid data in children during middle childhood (Schonert-Reichl, 1999; Ladd, Herald-Brown, & Riser, 2008; Wentzel, et al., 2007).

**Peer nominations of peer acceptance.** Children’s level of acceptance by peers was assessed using the same nomination sociometric procedure used for obtaining measures of behaviours (see Appendix B-3). Students were provided with a roster of all participating classmates’ names and asked to circle the names of all of their classmates for whom they “would like to be in school activities with” (e.g., Oberle et al., 2009). The percentage of nominations each child received was computed by dividing the number of nominations received by the total number of participating students in the classroom, where a higher score denotes greater levels of peer acceptance (see Ladd, Birch & Buhs, 1999). Scores on this scale have been shown to have acceptable reliability (e.g., test-retest,  $r = .81$ ; Asher, Singleton, Tinsley, & Hymel, 1979) and predictive validity with young children (see Ladd & Coleman, 1993). For example, in a sample of kindergarten children the stability of children’s averaged sociometric ratings was .77 over a 5 to 6 month interval (Ladd et al., 1999).

**Teacher-rated student-teacher closeness.** Teachers' perceptions of the quality of relationship with their students were assessed using the closeness subscale of the Student-Teacher Relationship Scale (STRS; Pianta, 2001; Pianta & Hamre, 2001; see Appendix B-4). The STRS is a self-report measure of teacher-perceived relationships with individual students. The closeness subscale is comprised of 11 items that measure warmth and open communication in the teacher-student relationship (i.e., "I share an affectionate, warm relationship with this child"). Teachers rated how applicable each statement was to their relationship with a particular student. Responses ranged from, definitely does not apply (1) to definitely applies (5). Items were averaged to yield a mean score. Higher scores represent greater levels of student-teacher closeness. In terms of reliability, statistically significant test-retest correlations over a four week period and high internal consistency of the closeness scale have been established (Pianta, 2001). The STRS has demonstrated predictive and concurrent validity and is related to current and future academic and social functioning (Hamre & Pianta, 2001), behavioural adjustment, and peer- and teacher-relations in pre-kindergarten through the elementary grades (Birch & Ladd, 1998; Jerome, Hamre, & Pianta, 2009). The STRS has been used extensively in studies of preschool and elementary-age children (Birch & Ladd, 1998; Hamre & Pianta, 2001; Jerome et al., 2009).

**Salivary cortisol.** Salivary cortisol was collected using a plastic Salivette device with a sterile cotton swab held inside a plastic tube (Salimetrics™). Absolute levels of cortisol in saliva represent the metabolically active ("free") fraction of cortisol that is able to pass into saliva and are strongly correlated with serum cortisol levels (see Kirschbaum & Hellhammer, 1994; van Goozen et al., 1998). Thus, salivary cortisol represents a relatively non-invasive and inexpensive method for indexing daily patterns in salivary cortisol. To capture daily patterns of cortisol in a classroom setting, salivary cortisol samples were obtained from children while residing in their

classrooms three times a day (9am, 12pm, and 3pm) over four days (Tuesday to Friday) for a total of 12 samples per subject. Prior to each morning saliva collection (9am), children recorded what time they woke up that day. Children also recorded recent food intake prior to every sample collection (see Appendix B-5). Salivettes were immediately centrifuged (within 3 hours of collection) at 3,000 rpm for 5 min resulting in a clear supernatant of low viscosity. Salivary cortisol levels were measured using a commercial immunoassay with chemiluminescence detection (CLIA; IBL-Hamburg, Hamburg, Germany). The lower concentration limit of this assay was 0.44 nmol/liter; intra- and inter-assay coefficients of variance were less than 8%. Any sample over 50 nmol/liter was repeated (see Badrick et al., 2008; Badrick, Kirschbaum & Kumari, 2007). This method of daily salivary cortisol collection has been successfully implemented in numerous studies in school-age children in classrooms and day camp settings (e.g., Lupien et al., 2001; Murray-Close et al., 2008; Oberlander et al., 2006).

### **Mediation Analyses**

As discussed in the literature review, several approaches for testing hypotheses about mediation have been proposed (see MacKinnon et al., 2002 for an overview). Over the years, these methods have grown in sophistication yet the analytical choices that researchers tend to make when testing intervening variables remains out of step with the advances in mediation analyses. In accordance with recent efforts by Hayes (2009) to “nudge the field towards a more modern way of thinking about the analyses of intervening variable effects” (p. 408), three approaches to mediation tests that are sequentially more sophisticated were used to examine hypotheses of mediation; (1) the ‘causal steps’ approach, (2) the product of coefficients approach, also known as the Sobel test, and (3) the bootstrapping approach for simple and multiple indirect effects.

**The causal steps approach.** Hierarchical multiple regression was used to test the mediating effects of peer acceptance and teacher closeness on the association between daily patterns of salivary cortisol and peer- and teacher-reported social behaviours, controlling for age and gender. To examine this model, recommendations for testing mediation through the causal steps approach were followed (Baron & Kenny, 1986). First a series of independent hierarchical regressions were used to regress the dependent variables- social behaviour on the predictor variable- cortisol. Age and gender were entered in the first step followed by the predictor in the second step, and the mediating variable in the third step. Criteria for mediation according to the causal steps approach was met if the change in  $R^2$  in the second step was significant when the mediator was absent from the model (path  $a$ ), yet the change in  $R^2$  was not significant when the mediator was added to the model in the third step (path  $c'$ ; Mackinnon et al., 2002).

**Product of coefficient approach.** Recommendations for testing mediation through the product of coefficients approach were then followed (Baron & Kenny, 1986; MacKinnon et al., 2002; MacKinnon, Warsi, & Dwyer, 1995). The maximum likelihood estimates from the hierarchical regressions provided the necessary path coefficients of  $a$  and  $b$  and the estimated standard errors of  $a$  and  $b$ . If the  $z$  statistic for the ratio of  $ab$  to its estimated standard error using the Aroian version of the Sobel test was significant at the .05 level, the effect was said to be mediated (MacKinnon et al., 2002).

**Bootstrapping indirect effects.** Next, simple and multiple mediation effects were tested using the non-parametric bootstrapping procedure. This approach is designed to describe the confidence intervals of the indirect effects in a manner that makes no assumptions about the distribution of the indirect effects. First, each of the putative mediators was examined alone in a series of tests for simple mediation of the relationship between cortisol and teacher- and peer-

reported behaviours. Next, the simple indirect effects of both mediators as well as pair wise contrasts between the two mediators were examined in a series of multiple mediation models. For each of the peer- and teacher-reported behaviours, separate mediation models were specified and tested. Bootstrapping estimation of the indirect effect for all social behaviours was estimated with 95% bias-corrected confidence estimates based on 1,000 bootstrap samples (Preacher & Hayes, 2004, 2008). For all analyses, age and gender were entered as covariates. Gender was coded as 1 = boy and 2 = girl. A significant indirect effect was interpreted if zero was not contained within the 95% confidence intervals. The total indirect effect was not reported as specific indirect effects that are in opposite directions may 'cancel out' yielding a total indirect effect that is small even when the specific indirect effects are large (Preacher and Hayes, 2004). The specific indirect effects represent the unique contribution of each mediator to the model and are testing different hypotheses, i.e., whether each indirect effect is different from zero. Therefore the two mediators can have similar point estimates (specific indirect effects) but only one of them may be statistically significant. Bootstrap contrasts were run to test whether there was a significant difference in magnitude between the two mediators (i.e., the indirect effects were unequal). As bootstrapping tests are sensitive to multi-collinearity, all regression models were examined for multi-collinearity using the criteria recommended by Tabachnick and Fidell (2001) that includes a Tolerance level  $< 2$ , Variance Inflation Factor  $< 4$ , and Condition Index  $< 15$ . The SPSS routines for bootstrap-based inferences were provided by Preacher and Hayes (2004).

## RESULTS

### Data Screening

The data were analyzed by first screening the data for accuracy of entry, patterns of missing data, and assumptions of multivariate analysis (normality, linearity, homoscedasticity, and independence). Scores that appeared deviant or out of range were checked with the questionnaire and corrected if warranted (< 1%). The distributions of all behavioural and relational variables appeared normal and residuals appeared to have a straight-line relationship with the predicted dependent variable scores. Normality was assessed by examining skew and kurtosis values and visually examining histograms and box plots of the data for outliers. Data were considered to exhibit univariate skew or kurtosis if the statistics equaled or exceeded  $|2.00|$  (Miles & Shevlin, 2001). Teacher-rated proactive aggression was found to demonstrate a positive skew indicating that teachers tended to rate students low in proactive aggression, which is expected in typical elementary classrooms. Teacher-rated proactive aggression was normalized following log<sub>10</sub> transformation and all analyses were performed using transformed values of teacher-rated proactive aggression.

Univariate outliers on the behavioural and relational variables were identified by examining box plots, minimum and maximum values, and as cases having very large standardized scores (approaching 4.0; Stevens, 1996). While a few of the behavioural variables had some cases outside of the whiskers of the box plot, in all of these cases the values were very close to the neighbouring values in the distribution and were within the normal expectations for scores on that variable. Essentially, no wild values or true outliers were identified using these methods. The cortisol data were screened to ensure each case had complete data for cortisol samples and 'time since wakening' at all time points. Single missing cortisol values (< 6%) were replaced by the day average of that time point for that particular child (see Popma et al., 2007a).

Outlier values were “winsorized” to within 3 *SD* of the mean (Gunnar, Mangelsdorf, Larson, & Hertsgard, 1989) following the method of Tukey (1977) and retained for data analysis. The term winsorize describes a general procedure whereby the researcher decides a priori on the values of outliers that will be brought closer to the normal distribution and is a procedure commonly applied to salivary cortisol data (e.g., Grunau et al., 2007; Wilcox, 1996). Consistent with previous studies, winsorized cortisol values exhibited a positive skew and were transformed to the natural logarithm scale (e.g., El-Sheik et al., 2008; Loney et al., 2006). A Kolmogorov–Smirnov analysis confirmed that the transformed cortisol values were normally distributed and all subsequent analyses were performed using log transformed cortisol values. To ease interpretation, untransformed mean cortisol values ( $\mu\text{g/dl}$ ) are displayed in Table 1 and Figure 4 (e.g., Lisonbee et al., 2008).

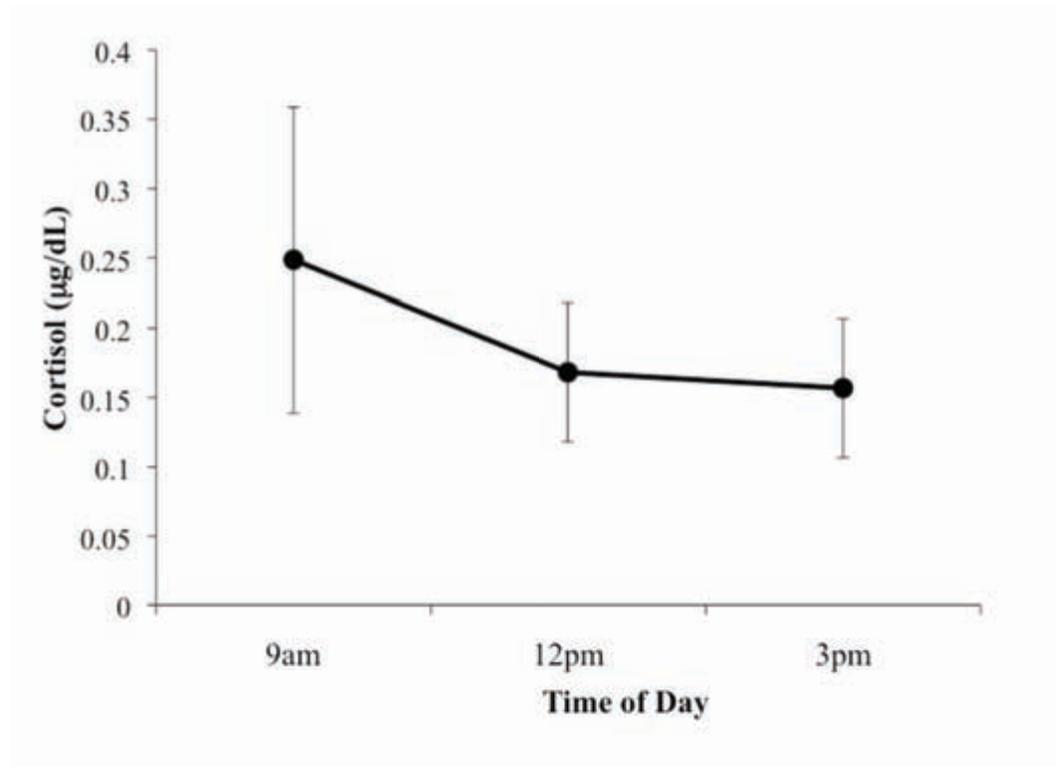
Multivariate outliers were examined using Mahalanobis’ distance. The standard method for multivariate outlier detection is robust estimation of the parameters in the Mahalanobis distance and the comparison with a critical value of the  $\chi^2$  distribution (Rousseeuw & van Zomeren, 1990). Mahalanobis’ distance values for each case were calculated by running a multiple regression with subject number as the dependent variable, the predictor variables as independent variables, and saving the resulting Mahalanobis’ distance for each case as a new variable. Separate, independent regressions were run for the cortisol variables, peer behaviours, teacher behaviours, and relational protective factor variables. These distances were then examined using descriptive statistics to see if any of the values were above the critical value, indicating that the case was a multivariate outlier. Critical values were identified as those higher than the  $\chi^2$  value with degrees of freedom equal to the number of variables in the analysis at  $p < .001$  (Tabachnick & Fidell, 2001). Bivariate plots of the relationships between the variables

Table 1. Descriptive statistics, skew, kurtosis, and scale reliabilities

Variable Name	<i>M</i>	<i>SD</i>	Skew	Kurtosis	$\alpha$	<i>N</i>	Min. to Max.
Age (years)	10.43	.62	.44	.54	n/a	89	9.26 to 12.18
Supportive Relationships							
Teacher Closeness	3.48	.81	-.18	-.79	.84	89	1.45 to 4.82
Peer Acceptance	.27	.12	-.03	-.14	n/a	88	.0 to .54
Teacher-reported Behaviours							
Prosocial	2.16	.54	-.29	-.99	.94	89	1 to 3
Reactive	1.42	.58	1.24	.32	.86	89	1 to 3
Proactive <sup>a</sup>	1.15	.24	2.37	5.79	.84	89	1 to 2.1
Social Aggression	1.35	.39	1.10	.87	.85	89	1 to 2.6
Peer-nominated Behaviours							
Prosocial	.59	.18	-.13	-.74	.89	88	.18 to .96
Reactive	.28	.21	.73	.46	.89	88	.0 to .94
Proactive	.19	.18	.63	-.31	.90	88	.0 to .73
Social Aggression	.32	.17	-.21	-.45	.70	88	.0 to .64
Cortisol <sup>b</sup>							
Morning (9am)	.25	.11	1.27	1.97	.86	89	.07 to .64
Noon (12pm)	.17	.05	.60	.83	.74	89	.07 to .33
Afternoon (3pm)	.16	.05	.71	.53	.64	89	.07 to .33
Diurnal Slope <sup>c</sup>	-.04	.03	-.33	1.46	-	89	-.13 to .07
AUC <sub>g</sub>	-4.74	.63	-.28	.29	-	89	-6.42 to -3.21
AUC <sub>i</sub>	-.80	.89	-.49	.27	-	89	-3.15 to -.80
Average Time of Awakening	7:30am	30 mins	-.29	-.34	-	89	6am to 8:30am

Note: <sup>a</sup>Values shown are prior to logarithmic transformation to correct skew. <sup>b</sup>Cortisol values indicate mean of four days in  $\mu\text{g/dl}$  prior to logarithmic transformation to correct skew. <sup>c</sup>Slope values represent cortisol values following logarithmic transformation.

Figure 4. Mean daily cortisol values across four consecutive days



were examined to assess the assumptions of linearity and homoscedasticity, and a logical consideration of whether the data met the assumptions of independence of observations was made.

Results demonstrated that the peer-nominated behaviours exceeded the critical value for Mahalanobis' distance,  $\chi^2(4) = 20.5$ , with one case identified as a multivariate outlier. Examination of the data confirmed no data entry error. This case scored maximum values on aggressive behaviours and minimum values on prosocial behaviours, but did not significantly differ on any of the demographic variables (e.g., age, family variables, and first language learned at home). These behavioural scores are conceptually sound suggesting that the case was sampled from the target population. According to recommendations by Tabachnick and Fidell (2001), the case was a true multivariate outlier on more than two peer-nominated behavioural variables and so the scores on the variables for the outlying case were not changed (i.e., made less deviant). All subsequent analyses involving peer-nominated behavioural variables were therefore examined with, and without, the multivariate outlier. Consistent with recommendations by Tabachnick and Fidell (2001), and Judd and McClelland (1989), a logical decision was made to drop the case from the analyses when found to significantly influence the results.

### **Data Reduction: Salivary Cortisol**

Average transformed cortisol values from across the four testing days were calculated to produce morning (9am), noon (12pm), and afternoon (3pm) basal values. As an indication of change in cortisol across the day, the average slope across four days, controlling for time since awakening was calculated (see Miller et al., 2007). To calculate slope, a linear regression was performed with transformed cortisol as the dependent variable and time since awakening as the independent variable for each day. The output was then organized by case and the coefficient statistics were saved to provide an estimation of slope. The procedure was repeated for each day

and the data were merged with the main dataset for an estimation of average slope for each child. Area under the curve with respect to ground (AUC<sub>g</sub>) and area under the curve with respect to increase (AUC<sub>i</sub>) were calculated as indicators of total cortisol output and rate of change of cortisol across the day, respectively (Fekedulegn et al., 2007; Pruessner et al., 2003).

### **Descriptive Statistics**

Means, standard deviations, and the possible range of scores were calculated for all variables and are reported in Table 1. Descriptive statistics for teacher closeness were moderately high indicating that, on average, teachers' rated their relationships with children as positive, which is consistent with values obtained in similar studies with children in grades 4 to 6 (e.g., Jerome et al., 2009) and kindergarten (Baker, Grant, & Morlock, 2008; Ladd & Burgess, 2001). The scores for peer acceptance were consistent with longitudinal research in sixth to eighth grade students (see Wentzel & Caldwell, 1997). The means and standard deviations for teacher- and peer-reported prosocial behaviours were moderately high, whereas the aggressive behaviours were relatively low. This behavioural profile is consistent with previous research with a non-clinical population of preadolescents (e.g., Veenstra et al., 2008). For example, teacher-reported prosocial behaviour values were consistent with those obtained in previous longitudinal work in children aged 10 to 14 (Nantel-Vivier et al., 2009). Peer-nominated social aggression values were shown to be; similar to values obtained in a large Canadian longitudinal study (N = 1,401) of 10 year olds (Vaillancourt et al., 2007), slightly lower than that obtained by Murray-Close, Ostrov and Crick (2007) in a longitudinal study during middle childhood, and higher than peer-nominated behavioural scores obtained at a week-long summer day camp where the children were not as familiar with each other (Murray-Close et al., 2008). All of these behavioural and relational values are consistent with what would be expected from a typical

elementary classroom sampled during Spring semester when children and teachers are relatively familiar to one another.

The means and standard deviations for daily cortisol values were consistent with a typical everyday pattern with highest cortisol observed in the morning ( $M = .25$ ;  $SD = .11$ ), followed by a steep decline to noon ( $M = .17$ ;  $SD = .05$ ), and a gradual decline in the afternoon ( $M = .16$ ;  $SD = .05$ ). All mean cortisol values reported are non-transformed values measured in  $\mu\text{g/dl}$  (see Table 1). To facilitate intra-study comparison, as recommended by Jessop and Turner-Cobb (2008) in an extensive review of cortisol measurement in children, the morning cortisol values converted to  $\text{nmol/l}$  ( $M = 6.9$ ;  $SD = 3.1$ ;  $Range = 1.94$  to  $17.76$   $\text{nmol/l}$ ) were found to be consistent with numerous other studies of 9am basal cortisol values in 8 to 11 year olds (see Jessop & Turner-Cobb, p. 3). Similar morning, noon, and afternoon cortisol values were obtained in previous research examining daily patterns of cortisol in middle childhood (e.g., Cicchetti et al., 2010; Murray-Close et al., 2008; Popma et al., 2007a).

Certain medications, medical diagnoses, and time of eating are known to affect salivary cortisol production in children and were documented prior to each saliva collection (see Hanrahan, McCarthy, Kleiber, Lutgendorf, & Tsalikian, 2006 for an overview of clinical methods for collecting salivary cortisol in children). Of the 12 students self-reporting a medical condition, 1 (1%) indicated diabetes; 1 (1%) attention deficit hyperactivity disorder but no medication; 6 (7%) with asthma, with only 1 student indicating regular use of an inhaler; and 4 (9%) reported 'other', which included 2 children with eczema, 1 with learning difficulties, and 1 with epilepsy taking regular but unspecified medication. A series of hierarchical regression analyses controlling for age and gender indicated no significant effect of medications or diagnoses of medical condition on morning, noon, and afternoon cortisol. Children reporting a medical condition did not differ from the rest of the sample on any indices of cortisol, behaviour,

and supportive relationships. Examination of the individual daily cortisol values across the four days for the student who reported epilepsy indicated values consistent with the rest of the sample, therefore this student's data was included in the analyses. Examination of the effect of food on cortisol levels for each day indicated no significant associations between eating breakfast, a snack at morning recess, and eating lunch / snack after lunch on morning, noon, and afternoon cortisol, respectively. Students' average wake up time across the four days significantly predicted: average morning cortisol,  $F(1, 85) = 16.31, p < .001$ ; noon cortisol,  $F(1, 85) = 7.45, p < .01$ , and AUCi,  $F(1, 85) = 26.12, p < .001$ . No significant effect of time of awakening was observed for afternoon cortisol,  $F(1, 85) = .64, p = .42$ , and AUCg,  $F(1, 85) = .001, p = .98$ . All regression analyses controlled for age and gender. To adjust morning and noon cortisol to a projected value for time since awakening, morning and noon cortisol values were individually regressed on time since awakening for each day, controlling for age and gender, and the residual values were used as predictors in all analyses. The number of hours since awakening at 3pm was included as a covariate in the analyses of multiple mediation with no difference in significance in findings. Similarly, time of awakening did not significantly influence regression analyses with AUCi. Therefore all analyses with afternoon cortisol and AUCi do not include adjustment for time since awakening. Previous research in adults has shown that time since awakening does not influence average cortisol values obtained over the course of a working day (e.g., Kunz-Ebrecht, Kirschbaum, & Steptoe, 2004).

A series of one-way analyses of variance (ANOVAs) were conducted to examine the influence of age, gender, and English as a second language (defined as first language learned at home) on the cortisol, behavioural, and relational variables. The results indicated that age and gender significantly influenced the majority of behavioural and relational variables and thus were included as covariates in subsequent regression analyses. Gender did not significantly

influence morning, noon, or afternoon basal cortisol values, and older children demonstrated higher afternoon cortisol,  $F(1, 86) = 6.62, p = .01$ . Irrespective of these findings, previous research has shown a significant influence of gender and age on measures of children's salivary cortisol across the day (e.g., Booth, Granger, & Shirtcliff, 2008; Shirtcliff et al., 2005), therefore all subsequent analyses controlled for age and gender. English as a second language demonstrated no significant relationship with all variables and thus was excluded from further analyses. All analyses were at the individual level, however to control for potential effects of classroom environment at the classroom level, a dummy variable for each of the four classrooms was included as a covariate in all multiple mediation analyses.

### **Correlations**

Pearson product-moment correlations were calculated among all variables of interest and are presented in Table 2. Caution must be taken with direct interpretation of these results due to the relatively small sample size and increased probability of Type 1 error due to multiple comparisons. Thus, the correlational results are presented as a conceptual illustration of the hypothesized directions of association among all variables. Tests of hypotheses were explored using regression analyses. The results demonstrated that age and gender were modestly correlated with many of the variables,  $r(88) = .21$  to  $.43$ , confirming that age and gender should be controlled for in the regression analyses. Examination of the association between various indicators of daily patterns of cortisol (i.e., morning, noon, afternoon cortisol, diurnal slope, AUCg, and AUCi) suggested that only afternoon (3pm) cortisol was significantly associated with the majority of behavioural and relational variables. Afternoon cortisol demonstrated moderate inverse correlations with the majority of aggressive behavioural variables,  $r(88) = -.22$  to  $-.38$  and positive correlations with the prosocial behavioural variables,  $r(88) = .33$  to  $.51$ . As an illustration of this finding, afternoon cortisol demonstrated the strongest positive correlation

Table 2. Pearson product-moment correlations among all variables

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1. Sex	-															
2. Age	.08	-														
Peer-nominated behaviour																
3. Prosocial	.43*	.31*	-													
4. Proactive	-.29*	-.20	-.47*	-												
5. Reactive	-.22*	-.37*	-.59*	.69*	-											
6. Social aggression	.11	-.13	-.27*	.66*	.59*	-										
Teacher-report behaviour																
7. Prosocial	.33*	.30*	.69*	-.46	-.46*	-.29*	-									
8. Proactive	-.08	.11	-.52*	.62*	.49*	.47*	-.54*	-								
9. Reactive	-.21*	-.11	-.54*	.66*	.66*	.40*	-.46*	.60*	-							
10. Social aggression	.07	.15	-.32*	.46*	.42*	.42*	-.22*	.59*	.50*	-						
Supportive Relationships																
11. Peer Acceptance	-.06	.32*	.64*	-.26*	-.41*	-.32*	.45*	-.26*	-.43*	-.19	-					
12. Teacher Closeness	.36*	.27*	.50*	-.32*	-.21*	-.12	.70*	-.35*	-.16	.04	.26*	-				

Note:  $N = 88$  for peer-nominated behaviours;  $N = 89$  for teacher-rated behaviours.

\*  $p < .05$

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
<b>Cortisol</b>																	
13. Morning	-.07	.07	-.04	.06	.08	-.02	.07	.12	.03	-.04	.05	.08	-				
14. Noon	.14	.18	.03	-.13	-.12	-.14	.09	.06	-.13	.03	-.02	.05	.23*	-			
15. Afternoon	.16	.28*	.33*	-.38*	-.38*	-.31*	.51*	-.09	-.33*	-.04	.34*	.41*	.25*	.36*	-		
16. Slope	.22*	.09	.26*	-.29*	-.29*	-.19	.27*	-.19	-.24*	-.01	.15	.22*	-.70*	.09	.44*		
17. AUCg	.07	.18	.05	-.06	-.06	-.13	.17	.05	-.08	.03	.13	.16	.71*	.78*	.53*	-.26*	
18. AUCi	.17	.02	.10	-.18	-.12	-.08	.04	-.12	-.13	.02	-.06	.01	-.75*	.23*	.08	.83*	-.30*

Note:  $N = 88$  for peer-nominated behaviours;  $N = 89$  for teacher-rated behaviours.

\*  $p < .05$

with teacher-rated prosocial behaviour,  $r(89) = .51$ , and strongest negative correlation with peer-nominated proactive and reactive aggression,  $r(88) = -.38$ . Afternoon cortisol demonstrated moderate positive associations with the putative mediators, teacher closeness,  $r(89) = .41$  and peer acceptance,  $r(88) = .34$  which may lead to collinearity issues in the regression analyses. Consistent with the suggestion that supportive relationships with peers and teachers are associated with prosocial behaviours, moderate to strong positive correlations were observed with prosocial behaviours,  $r(88) = .45$  to  $.70$ , whereas inverse association were observed with aggressive behaviours,  $r(88) = -.21$  to  $-.43$ . The teacher- and peer-reports of children's aggressive behaviours demonstrated moderate inter-correlations,  $r(89) = .50$  to  $.60$ , and  $r(88) = .59$  to  $.69$ , respectively suggesting that they may have similar underlying constructs, a phenomenon discussed in the literature (see Crapanzano et al., 2010; Little et al., 2003). Teacher reported social aggression was the only behaviour not significantly correlated with afternoon cortisol or the supportive relationships however this finding will be further explored in regression analyses. Both cortisol slope and afternoon cortisol demonstrated similar directions of correlations with the behavioural and relational variables, with cortisol slope demonstrating smaller correlations,  $r(88) = -.22$  to  $-.29$ . Overall these correlation patterns are consistent with theoretically expected relationships and will be examined further in a series of regression analyses.

### **Tests of Hypotheses**

In this section, all results are presented in tables instead of path diagrams for ease of interpretation. The results are organized into four sections: (1) main effects examining the association between cortisol and peer- and teacher-reported behaviours (path  $c$ ); simple indirect effects (path  $c'$ ) of the individual mediators, (2) peer acceptance, and (3) teacher closeness; and (4) multiple indirect effects that control for the presence of both mediators. Simple indirect

effects of each mediator were calculated using three approaches to mediation (i.e., causal paths, product of coefficient, and bootstrapping of indirect effects) and are presented with the objective of comparing the results of each methodological approach. Given the robust nature of the bootstrapping approach (see Preacher & Hayes, 2008), the data were interpreted based on results of bootstrapping the multiple indirect effects. For ease of presentation, the results section is formatted according to each mediator. The focus of the discussion will return to the dependent outcome social behaviour and thus will be formatted according to each subtype of behaviour.

**Main effects (path *c*).** The first hypothesis of the study was that peer- and teacher-reported reactive aggression, proactive aggression, and social aggression would be significantly associated with daily patterns of salivary cortisol. Various indicators of daily patterns in salivary cortisol were explored including, cortisol at specific times in the day (i.e., morning, noon, afternoon), change in cortisol across the day (i.e., morning to afternoon diurnal slope), total daily output (AUC<sub>g</sub>), and rate of change of cortisol across the day (AUC<sub>i</sub>). A series of independent hierarchical linear regressions were conducted with behaviour as the dependent variable and cortisol as the independent variable. Age and gender were entered in the first step as covariates followed by cortisol in the second step. The results of the regression analyses for the different indicators of cortisol are found in Tables 3 to 8. The findings suggest no significant association of morning cortisol, noon cortisol, AUC<sub>g</sub>, or AUC<sub>i</sub> to peer- and teacher-reported behaviours (see Tables 3, 4, 7 and 8). Higher afternoon cortisol was significantly associated with greater peer- and teacher-reported prosocial behaviour, lower levels of peer-nominated proactive, reactive, and social aggression, and teacher-reported reactive aggression (see Table 5). No significant association was observed between afternoon cortisol and teacher-reported socially aggression. Steeper cortisol slope (i.e., higher morning and lower afternoon cortisol) was significantly associated with peer-nominated reactive, proactive, and social aggression (see Table 6).

Table 3. Association of morning cortisol to teacher- and peer-reported behaviours (path *c*).

	Morning Cortisol						
	<i>B</i>	<i>SEB</i>	$\beta$	<i>F(df)</i>	<i>p</i>	$\Delta R^2$	95% CI
Teacher-reported Behaviours							
Prosocial behaviour	.05	.07	.07	.55 (1, 85)	.46	.01	-.08, .18
Reactive aggression	.01	.08	.02	.03 (1, 85)	.87	.00	-.14, .16
Proactive aggression	.01	.01	.11	1.08 (1, 85)	.30	.01	-.01, .02
Social aggression	-.02	.05	-.04	.16 (1, 85)	.69	.00	-.13, .08
Peer-nominated Behaviours							
Prosocial behaviour	-.01	.02	-.03	.09 (1, 84)	.76	.00	-.05, .04
Reactive aggression	.02	.03	.09	.84 (1, 84)	.36	.01	-.03, .07
Proactive aggression	.01	.02	.05	.25 (1, 84)	.62	.00	-.03, .06
Social aggression	.01	.02	.04	.11 (1, 84)	.74	.00	-.04, .05

Note: *N* = 89 for teacher-reported behaviours; *N* = 88 for peer-reported behaviours.

CI = Confidence intervals, lower and upper limits.

\**p* < .05

Table 4. Association of noon cortisol to teacher- and peer-reported behaviours (path *c*).

	Noon Cortisol						
	<i>B</i>	<i>SEB</i>	$\beta$	<i>F(df)</i>	<i>p</i>	$\Delta R^2$	95% CI
Teacher-reported Behaviours							
Prosocial behaviour	-.001	.07	-.002	.00 (1, 85)	.98	.00	-.15, .14
Reactive aggression	-.07	.09	-.09	.72 (1, 85)	.40	.01	-.24, .10
Proactive aggression	.004	.01	.05	.23 (1, 85)	.63	.003	-.01, .02
Social aggression	-.004	.06	-.01	.01 (1, 85)	.94	.000	-.12, .11
Peer-nominated Behaviours							
Prosocial behaviour	-.02	.02	-.09	.82 (1, 84)	.37	.01	-.07, .03
Reactive aggression	-.01	.03	-.02	.06 (1, 84)	.82	.001	-.06, .05
Proactive aggression	-.02	.03	-.06	.34 (1, 84)	.56	.004	-.07, .04
Social aggression	-.02	.03	-.09	.74 (1, 84)	.39	.01	-.07, .03

Note: *N* = 89 for teacher-reported behaviours; *N* = 88 for peer-reported behaviours.

CI = Confidence intervals, lower and upper limits.

\**p* < .05

Table 5. Association of afternoon cortisol to teacher- and peer-reported behaviours (path *c*).

	Afternoon Cortisol						
	<i>B</i>	<i>SEB</i>	$\beta$	<i>F(df)</i>	<i>p</i>	$\Delta R^2$	95% CI
Teacher-reported Behaviours							
Prosocial behaviour	1.79	.38	.43*	21.64 (1, 85)	.00	.17	1.02, 2.56
Reactive aggression	-1.35	.48	-.30*	7.99 (1, 85)	.01	.08	-2.30, -.39
Proactive aggression	-.05	.05	-.12	1.11 (1, 85)	.30	.01	-.15, .04
Social aggression	-.29	.34	-.09	.69 (1, 85)	.41	.01	-.96, .41
Peer-nominated Behaviours							
Prosocial behaviour	.28	.13	.20*	4.40 (1, 84)	.04	.04	.01, .54
Reactive aggression	-.44	.16	-.32*	7.76 (1, 84)	.01	.09	-.76, -.19
Proactive aggression	-.44	.14	-.32*	9.62 (1, 84)	.003	.09	-.71, -.16
Social aggression	-.42	.14	-.33*	9.17 (1, 84)	.003	.10	-.70, -.15

Note: *N* = 89 for teacher-reported behaviours; *N* = 88 for peer-reported behaviours.

CI = Confidence intervals, lower and upper limits. VIF was 1.05 for all analyses

\**p* < .05

Table 6. Association of diurnal cortisol slope to teacher- and peer-reported behaviours (path *c*).

	Cortisol Diurnal Slope					
	<i>B</i>	<i>SEB</i>	$\beta$	<i>F(df)</i>	<i>p</i>	95% <i>CI</i>
Teacher-reported Behaviours						
Prosocial	3.16	1.67	.19	3.59 (1, 85)	.06	-.16, 6.48
Reactive aggression	-3.34	1.93	-.20	3.53 (1, 85)	.06	-7.48, .21
Proactive aggression	-.32	.19	-.19	2.91(1, 85)	.09	-.69, .05
Social aggression	-.38	1.36	-.03	.08 (1, 85)	.78	-3.08, 2.33
Peer-reported Behaviours						
Prosocial aggression	.89	.53	.16	2.84 (1, 84)	.10	-.16, 1.93
Reactive aggression	-1.47	.63	-.23*	5.40 (1, 84)	.02	-2.73, -.21
Proactive aggression	-1.26	.57	-.23*	4.90 (1, 84)	.03	-2.39, -.13
Social aggression	-1.15	.57	-.22*	4.07 (1, 84)	.047	-2.28, -.02

Note: *N* = 89 for teacher-reported behaviours; *N* = 88 for peer-reported behaviours.

*CI* = Confidence intervals, lower and upper limits. VIF was < 2.00 for all analyses

\**p* < .05

Table 7. Association of AUCg cortisol to teacher- and peer-reported behaviours (path *c*).

	AUCg Cortisol						
	<i>B</i>	<i>SEB</i>	$\beta$	<i>F(df)</i>	<i>p</i>	$\Delta R^2$	95% CI
Teacher-reported Behaviours							
Prosocial behaviour	.21	.20	.11	1.12 (1, 85)	.29	.01	-.18, .59
Reactive aggression	-.12	.23	-.05	.26 (1, 85)	.61	.003	-.57, .34
Proactive aggression	.02	.02	.10	.82 (1, 85)	.37	.01	-.02, .06
Social aggression	.002	.16	.002	.00 (1, 85)	.99	.00	-.31, .31
Peer-nominated Behaviours							
Prosocial behaviour	-.02	.06	-.03	.13 (1, 84)	.73	.001	-.14, .10
Reactive aggression	.01	.08	.01	.02 (1, 84)	.90	.00	-.14, .16
Proactive aggression	-.01	.07	-.01	.01 (1, 84)	.92	.00	-.14, .13
Social aggression	-.06	.07	-.09	.67 (1, 84)	.41	.01	-.19, .08

Note: *N* = 89 for teacher-reported behaviours; *N* = 88 for peer-reported behaviours.

CI = Confidence intervals, lower and upper limits.

\**p* < .05

Table 8. Association of AUCi cortisol to teacher- and peer-reported behaviours (path *c*).

	AUCi Cortisol						
	<i>B</i>	<i>SEB</i>	$\beta$	<i>F(df)</i>	<i>p</i>	$\Delta R^2$	95% CI
Teacher-reported Behaviours							
Prosocial behaviour	-.02	.11	-.02	.03 (1, 85)	.86	.00	-.23, .19
Reactive aggression	-.12	.12	-.10	.87 (1, 85)	.35	.01	-.36, .13
Proactive aggression	-.02	.01	-.14	1.56 (1, 850)	.22	.02	-.04, .01
Social aggression	.01	.09	.01	.01 (1, 85)	.94	.00	-.16, .18
Peer-nominated Behaviours							
Prosocial behaviour	.01	.03	.03	.11 (1, 84)	.74	.00	-.06, .08
Reactive aggression	-.04	.04	-.09	.73 (1, 84)	.40	.01	-.12, .05
Proactive aggression	-.05	.04	-.14	1.71 (1, 84)	.19	.02	-.12, .03
Social aggression	-.04	.04	-.12	1.24 (1, 840)	.27	.01	-.11, .03

Note: *N* = 89 for teacher-reported behaviours; *N* = 88 for peer-reported behaviours.

CI = Confidence intervals, lower and upper limits.

\**p* < .05

No significant associations were observed between cortisol slope and prosocial behaviours or teacher-reported, proactive, reactive, and social aggression. Follow-up analyses standardizing teacher reports of behaviour to each classroom, and testing for possible influence of different classrooms (dummy variable for each of the 4 classrooms as a covariate) did not alter the significance of these results. The findings suggest that daily patterns of salivary cortisol are associated with peer- and teacher- reports of children's proactive, reactive, and social aggression and prosocial behaviour in a typical classroom setting. Afternoon cortisol was more strongly associated with children's social behaviour compared to diurnal slope suggesting that daily patterns of cortisol may be more strongly related to behaviour during specific periods of the cortisol diurnal rhythm.

**Simple mediation.** The second hypothesis predicted that peer acceptance and teacher closeness would uniquely mediate the association between cortisol and peer- and teacher-reports of children's social behaviours. To investigate this hypothesis, each of the putative mediators were individually examined in series of tests for simple mediation, first using the causal steps approach, followed by the Sobel test, and finally bootstrapping of simple indirect effects. The Sobel test and bootstrapping results for peer-nominated reactive and social aggression were significantly influenced by a multivariate outlier on peer-nominated behaviours. Therefore, all results for peer-nominated reactive and social aggression are reported with the outlier removed (see Appendix D for the unstandardized regression coefficients). Results of a series of hierarchical regression analyses revealed a non-significant association of numerous indicators of daily cortisol (i.e., morning, noon, diurnal slope, AUC<sub>g</sub>, and AUC<sub>i</sub>) to the mediators, peer acceptance and teacher closeness (path *a*; see Table 9). A non-significant association between an independent variable and putative mediators does not meet criteria for mediation, therefore

Table 9. Associations among daily patterns of salivary cortisol to peer acceptance and teacher closeness (path *a*).

	<i>B</i>	<i>SEB</i>	$\beta$	<i>F(df)</i>	<i>p</i>	95% CI
Morning Cortisol						
Teacher Closeness	.07	.08	.09	.77 (1, 85)	.38	-.09, .24
Peer Acceptance	.004	.02	.02	.06 (1, 84)	.82	-.03, .03
Noon Cortisol						
Teacher Closeness	-.04	.09	-.04	.14 (1, 85)	.71	-.22, .15
Peer Acceptance	-.01	.02	-.03	.09 (1, 84)	.77	-.04, .03
Afternoon Cortisol						
Teacher Closeness	1.97	.60	.32*	10.65 (1, 85)	.002	.77, 3.17
Peer Acceptance	.27	.10	.29*	7.82 (1, 84)	.01	.08, .46
Cortisol Slope						
Teacher Closeness	3.33	2.52	.13	1.76 (1, 85)	.19	-1.67, 8.33
Peer Acceptance	.53	.39	.14	1.85 (1, 84)	.18	-.25, 1.31
AUCg						
Teacher Closeness	.24	.25	.10	.90 (1, 85)	.35	-.26, .74
Peer Acceptance	.04	.05	.08	.61 (1, 84)	.44	-.06, .13
AUCi						
Teacher Closeness	-.06	.14	-.04	.15 (1, 85)	.66	-.33, .21
Peer Acceptance	-.01	.03	-.05	.27 (1, 84)	.60	-.06, .04

*N* = 89 for teacher-reported behaviours; *N* = 88 for peer-reported behaviours.

*CI* = Confidence intervals, lower and upper limits. VIF was < 2.00 for all analyses

\**p* < .05

these indicators of cortisol were not explored in analyses of mediation. All further analyses are discussed with reference to afternoon cortisol as the predictor.

**Peer acceptance.** To investigate the hypotheses that peer acceptance mediates the association between cortisol and peer- and teacher-reported social behaviours, a series of hierarchical linear regressions were run for each individual behaviour. Age and gender were entered in the first step followed by afternoon cortisol in the second step and peer acceptance in the third step to give the necessary path coefficients for path *a*, path *b* and path *c*'.

**Causal path approach.** Results of a series of regression analyses examining the association of afternoon cortisol to the mediators, peer acceptance and teacher closeness, indicated that higher afternoon cortisol was significantly associated with higher ratings of the mediator, peer acceptance (path *a*; Table 9). Regression analyses examining the association of peer acceptance and teacher closeness to behaviour (path *b*), controlling for afternoon cortisol indicated that peer acceptance was positively associated with peer- and teacher-reported prosocial behaviour, and inversely associated with peer- and teacher-reported reactive aggression and teacher-reported social aggression (see Table 10). Peer acceptance was not significantly associated with peer and teacher reported proactive aggression or peer-reported social aggression when the multivariate outlier case for peer behaviours was removed. Next, a series of hierarchical regression analyses examined whether there was a significant mediating influence of peer acceptance on the association between afternoon cortisol and social behaviours (path *c*'; Table 11). When the putative mediator peer acceptance was included in the third step, the beta coefficients between afternoon cortisol and teacher- and peer rated prosocial and teacher-rated reactive aggressive behaviours were either no longer significant or substantially reduced (path *c*') meeting the conditions for mediation according to the causal path approach (see Baron & Kenny, 1986; see Table 11).

Table 10. Associations of peer acceptance and teacher closeness to teacher- and peer-reported behaviours, controlling for afternoon cortisol (path *b*).

Behaviours	Teacher Closeness						Peer Acceptance					
	<i>B</i>	<i>SEB</i>	$\beta$	<i>F(df)</i>	<i>p</i>	95% <i>CI</i>	<i>B</i>	<i>SEB</i>	$\beta$	<i>F(df)</i>	<i>p</i>	95% <i>CI</i>
Teacher-report												
Prosocial	.45	.06	.58*	51.70 (1, 84)	<.00	.33, .58	1.49	.42	.33*	12.96 (1, 83)	.001	.67, 2.32
Reactive aggression	.04	.10	.04	.13 (1, 84)	.72	-.16, .23	-1.97	.51	-.40*	15.06 (1, 83)	<.00	-2.99, -.96
Proactive aggression	-.09	.04	-.26*	4.87 (1, 84)	.03	-.17, -.01	-.02	.01	-.19	2.64 (1, 83)	.11	-.04, .00
Social aggression	.004	.07	.01	.004 (1, 84)	.95	-.14, .15	-.81	.39	-.25*	4.38 (1, 83)	.04	-1.58, -.04
Peer-report												
Prosocial	.08	.03	.30*	9.28 (1, 83)	.003	.03, .13	.96	.11	.64*	76.68 (1, 83)	<.00	.74, 1.18
Reactive aggression <sup>a</sup>	.01	.03	.04	.17 (1, 82)	.68	-.05, .07	-.33	.17	-.20*	3.88 (1, 82)	.05	-.66, -.00
Proactive aggression	-.02	.03	-.07	.38 (1, 83)	.55	-.08, .04	-.25	.16	-.17	2.39 (1, 83)	.13	-.56, .07
Social aggression <sup>a</sup>	.001	.03	.004	.001 (1, 82)	.98	-.06, .06	-.28	.16	-.20	2.99 (1, 82)	.09	-.61, .04

Note: *N* = 89 for teacher-reported behaviours; *N* = 88 for peer-reported behaviours except <sup>a</sup>reactive aggression and social aggression where *N* = 87. *CI* = Confidence intervals, lower and upper limits

\**p* < .05

Table 11. Comparison of causal steps approach, Sobel test, and bootstrapping simple and multiple mediation of the indirect effects of afternoon cortisol to social behaviours through changes in peer acceptance and teacher closeness (path  $c'$ ).

		Bootstrapping Indirect Effects ( $N = 1000$ )						
Behaviour	Teacher /peer-rated Mediator	Simple Mediation			Multiple Mediation			
		Causal Steps path $c$ (path $c'$ )	Sobel Test (Aroian)	Simple Mediator	Point Estimate (95% BCaCI)	Multiple Mediator	Point Estimate (95% BCaCI)	Contrast (95% BCaCI)
<b>Prosocial Behaviour</b>								
Teacher-rated								
	Peer Acceptance	.34*(.43*) Yes	2.16* Yes	Yes	.40 (.10, .94)	Yes	.31 (.06, .65)	.36 (-.17, .94)
	Teacher Closeness	.26*(.43*) Yes	2.89* Yes	Yes	.72 (.27, 1.30)	Yes	.67 (.28, 1.18)	
Peer-rated								
	Peer Acceptance	.02 (.20*) Yes	2.65* Yes	Yes	.25 (.05, .48)	Yes	.24 (.05, .45)	-.15 (-.36, .05)
	Teacher Closeness	1.03 (.20*) Yes	2.17* Yes	Yes	.14 (.04, .30)	Yes	.09 (.03, .20)	
<b>Reactive Aggression</b>								
Teacher-rated								
	Peer Acceptance	-.18 (-.30*) Yes	-2.22* Yes	Yes	-.53 (-1.10, -.08)	Yes	-.58 (-1.24, -.16)	.74 (.15, 1.54)*
	Teacher Closeness	-.31* (-.30*) No	.29 No	No	.05 (-.34, .47)	No	.15 (-.19, .63)	
Peer-rated <sup>a</sup>								
	Peer Acceptance	-.26* (-.32*) No	-1.54 No	No	-.09 (-.23, .002)	No	-.09 (-.23, .003)	.12 (-.05, .30)
	Teacher Closeness	-.32* (-.32*) No	.18 No	No	.01 (-.10, .12)	No	.02 (-.09, .15)	
<b>Proactive Aggression</b>								
Teacher-rated								
	Peer Acceptance	-.07 (-.12) No	-1.33 No	Yes	-.05 (-.13, .01)	Yes	-.04 (-.10, -.00)	.01 (-.06, .09)
	Teacher Closeness	-.11(-.21) No	-2.04* Yes	Yes	-.06 (-.14, -.02)	Yes	-.05 (-.13, -.01)	

		Bootstrapping Indirect Effects ( <i>N</i> = 1000)					
Behaviour Teacher /peer-rated Mediator			Simple Mediation		Multiple Mediation		
	Causal Steps path <i>c</i> (path <i>c'</i> )	Sobel Test (Aroian)	Simple Mediator	<i>Point Estimate (95% BCaCI)</i>	Multiple Mediator	<i>Point Estimate (95% BCaCI)</i>	<i>Contrast (95% BCaCI)</i>
<b>Proactive Aggression</b>							
Peer-rated							
Peer Acceptance	-.27* (-.32*) No	-1.29 No	No	-.07 (-.22, .01)	No	.06 (-.21, .01)	-.02 (-.19, .15)
Teacher Closeness	-.28* (-.32*) No	-.95 No	No	-.05 (-.17, .03)	No	-.04 (-.16, .04)	
<b>Social Aggression</b>							
Teacher-rated							
Peer Acceptance	-.02 (-.09) No	-1.61 No	Yes	-.22 (-.62, -.01)	Yes	-.22 (-.59, -.02)	.27 (-.03, .66)
Teacher Closeness	-.10 (-.09) No	.08 No	No	.009 (-.46, .23)	No	.04 (-.29, .30)	
Peer-rated <sup>a</sup>							
Peer Acceptance	-.27* (-.33*) No	-1.41 No	No	-.08 (-.24, .001)	No	-.08 (-.20, .01)	.07 (-.06, .23)
Teacher Closeness	-.32* (-.33*) No	-.38 No	No	-.02 (-.11, .07)	No	.01 (-.10, .08)	

Note: *N* = 89 for teacher-reported behaviours; *N* = 88 for peer-reported behaviours except <sup>a</sup> reactive aggression and social aggression where *N* = 87. Causal Steps path: Beta coefficients calculated prior to including the mediator in the model are in parentheses. *BCaCI*= bias corrected bootstrapping samples and confidence intervals that include corrections for both median bias and skew. Confidence intervals containing zero are interpreted as not significant. A negative point estimate means a decrease in levels of behaviour as afternoon cortisol increases. A positive point estimate means an increase in ratings of behaviour as afternoon cortisol increases. Yes = A statistically significant mediation effect was observed. \* *p* < .05;

*Product of coefficients approach.* Consistent with findings using the causal path approach, a follow up Sobel test (Aroian version) indicated that the indirect effect (path  $ab = c - c'$ ) was statistically significant for teacher-rated prosocial behaviour ( $z = 2.16$ ) and reactive aggression ( $z = -2.22$ ), and peer-nominated prosocial behaviour ( $z = 2.65$ ), but not peer-nominated reactive aggression ( $z = -1.54$ ). No significant indirect effect was observed for teacher- and peer-reported proactive aggression ( $z = -1.74$ ;  $z = -1.29$ ), and social aggression ( $z = -1.61$ ;  $z = -1.41$ ), respectively (see Table 11).

*Bootstrapping simple indirect effects.* Finally, significance of the simple indirect effects were formally tested through bootstrapping methods. The results suggest that the significance of the indirect effect for teacher- and peer-reported prosocial behaviours and teacher reported reactive aggression were similar to the point estimates computed from the conventional regression analyses according to the Baron and Kenny criteria (1986) with 95% confidence (see Table 11). Thus, the results of the causal steps approach and the bootstrapping test were in agreement that peer acceptance mediated the relationship between afternoon cortisol and teacher-rated prosocial behaviour, reactive aggression, and peer-nominated prosocial behaviours. However, inconsistent with the causal steps and the Sobel test approach, the bootstrapping of simple indirect effects indicated that peer acceptance was a significant mediator of the association between afternoon cortisol and teacher-reported proactive and social aggression. In summary, the results of bootstrapping of simple indirect effects revealed that; (1) lower afternoon cortisol predicts higher levels of teacher- and peer-reported reactive and social aggression through lower levels of peer acceptance; and (2) higher afternoon cortisol predicts increased ratings of teacher- and peer-reported prosocial behaviours through higher ratings of peer acceptance (see Table 11 and 12).

Table 12. Comparison of findings using different approaches to mediation analyses.

<b>Behaviour</b> Teacher/peer rated Mediator	Causal Steps path <i>c</i> (path <i>c'</i> )	Sobel Test (Aroian)	Simple Mediation	Multiple Mediation
<b>Prosocial Behaviour</b>				
Teacher-rated				
Peer Acceptance	Yes	Yes	Yes	Yes
Teacher Closeness	Yes	Yes	Yes	Yes
Peer-rated				
Peer Acceptance	Yes	Yes	Yes	Yes
Teacher Closeness	Yes	Yes	Yes	Yes
<b>Reactive Aggression</b>				
Teacher-rated				
Peer Acceptance	Yes	Yes	Yes	Yes
Teacher Closeness	No	No	No	No
Peer-rated <sup>a</sup>				
Peer Acceptance	No	No	No	No
Teacher Closeness	No	No	No	No
<b>Proactive Aggression</b>				
Teacher-rated				
Peer Acceptance	No	No	Yes	Yes
Teacher Closeness	No	Yes	Yes	Yes
Peer-rated				
Peer Acceptance	No	No	No	No
Teacher Closeness	No	No	No	No
<b>Social Aggression</b>				
Teacher-rated				
Peer Acceptance	No	No	Yes	Yes
Teacher Closeness	No	No	No	No
Peer-rated <sup>a</sup>				
Peer Acceptance	No	No	No	No
Teacher Closeness	No	No	No	No

Note: *N* = 89 for teacher-reported behaviours; *N* = 88 for peer-reported behaviours except <sup>a</sup>reactive aggression and social aggression where *N* = 87. Yes = A statistically significant mediation effect was observed.

**Teacher Closeness.** Similar to the analyses with the mediator peer acceptance, a series of hierarchical linear regressions were run for each individual behaviour, with age and gender entered in the first step, followed by afternoon cortisol in the second step, and teacher closeness in the third step to give the necessary path coefficients for path *a*, path *b* and path *c*'.

**Causal path approach.** Results indicated that higher afternoon cortisol was significantly associated with higher ratings of the mediator, teacher closeness (see Table 9). Teacher closeness was positively associated with peer- and teacher-reported prosocial behaviour, and negatively associated with teacher-reported proactive aggression (path *b*; see Table 10). No significant association was observed between teacher closeness to teacher-reported reactive and social aggression, and peer-reported reactive, proactive, and social aggression (Table 10). When the putative mediator, teacher closeness was included in step 3 of the regression analyses, the beta coefficients between afternoon cortisol and teacher- and peer rated prosocial behaviours were either no longer significant or substantially reduced (path *c*'; see Table 11) meeting the conditions for mediation using the causal path approach (see Baron & Kenny, 1986).

**Product of coefficient approach.** Consistent with findings using the causal path approach, a follow up Sobel test (Aroian version) indicated that the indirect effect path  $ab = c - c'$  of teacher closeness was statistically significant for teacher-rated prosocial behaviour ( $z = 2.89$ ) and peer-nominated prosocial behaviour ( $z = 2.17$ ; see Table 11). Consistent with the causal path approach, no significant indirect effect was observed for teacher- and peer-reported reactive aggression ( $z = .29$ ;  $z = .18$ , respectively), social aggression ( $z = .08$ ;  $z = -.38$ , respectively), or peer-nominated proactive aggression ( $z = -.95$ ). Inconsistent with the causal paths approach, a significant mediating influence of teacher closeness was observed for teacher-rated proactive aggression. While the direct association between afternoon cortisol and teacher-rated proactive aggression was statistically non-significant (i.e., path *c*), the observed significance of path *a* and

path *b*, combined with a reduction in cortisol beta coefficients following inclusion of teacher closeness (path *c'*) suggested a mediating relationship that was verified with the Sobel test ( $z = -2.04$ ). This inconsistency between the findings with the causal steps approach and Sobel test illustrates a limitation to the causal steps approach, where a significant association between the predictor and outcome variable (i.e., path *c*) is required for mediation.

*Bootstrapping simple indirect effects.* Formal testing of the simple indirect effects using the bootstrapping approach revealed consistent results with the Sobel test. Teacher closeness was shown to mediate the association between afternoon cortisol and teacher- and peer-reported prosocial behaviour, and teacher-reported proactive aggression (see Table 11).

**Multiple mediation.** Each of the putative mediators, peer acceptance and teacher closeness, were examined together in series of multiple mediation models to calculate bootstrapping estimates of specific indirect effects of afternoon cortisol to peer- and teacher-reported behaviours, controlling for age and gender (see Figure 3B). Bootstrap contrasts were run to test whether there was a significant difference in magnitude between the two mediators (i.e., the indirect effects were unequal). The point estimates and bias corrected 95% confidence intervals are presented in Table 11. No *p*-value is reported as bootstrapping is used for the generation of the asymmetric confidence interval for the indirect effect. All regression analyses were found to be within acceptable range of the assumptions of multi-collinearity.

Consistent with the proposed hypotheses, peer acceptance and teacher closeness were found to uniquely mediate the association between afternoon cortisol to peer- and teacher-reported behaviours. The mediating influence of both peer acceptance and teacher closeness for each of the peer- and teacher-reported behaviours is presented below (see Tables 11 and 12 for a summary of findings; see Appendix D for the unstandardized regression coefficients).

To examine the possible influence of time since awakening or classrooms effects on these results, the four classrooms were assigned a dummy variable and included as a covariate with age, and gender. Children's hours since time of awakening at 3pm was also included as a covariate. No difference in significance of findings was observed therefore the results are presented with only age and gender as covariates.

**Prosocial behaviour.** The results revealed significant specific indirect effects suggesting that teacher closeness and peer acceptance are unique mediators of prosocial behaviour, i.e., they contribute to the indirect effect above and beyond the presence of each other, age, and gender (see Table 11). The point estimates (and beta coefficients of path *a* and *b*; see Appendix D) were lying in a positive direction. This finding is consistent with the interpretation that higher afternoon cortisol leads to greater ratings of peer acceptance and teacher closeness, that in turn leads to higher levels of teacher- and peer-rated prosocial behaviours. From Table 11, it is expected that an increase in afternoon cortisol by one unit will increase teacher rated prosocial behaviour by .67 units via cortisol's effect on the mediator teacher closeness, and separately, by .31 units through the mediator peer acceptance. An increase in afternoon cortisol by one unit is expected to increase peer nominated prosocial behaviour by .09 units through the mediator teacher closeness; and separately, by .24 units through the mediator peer acceptance. These findings suggest that both peer acceptance and teacher closeness are unique mediators in the association between afternoon cortisol and prosocial behaviour. Pairwise contrasts indicated no significant difference in the magnitude of the unique effects of the two mediators for either teacher- or peer-rated prosocial behaviours.

**Subtypes of aggression.** The results indicated that lower afternoon cortisol leads to lower ratings of peer acceptance and teacher closeness, which in turn leads to higher levels of teacher- and peer-rated aggressive behaviours. For reactive aggression, the results were consistent with

those using the simple mediation approach and the Sobel test. Examination of the specific indirect effects indicated that peer acceptance was a significant and unique mediator of the effect of afternoon cortisol on teacher-reported reactive aggression controlling for teacher closeness, age, and gender (see Table 11). A point estimate of  $-.58$  for the specific indirect effect of peer acceptance suggests that as afternoon cortisol levels increased by one unit, teacher rated reactive aggression decreased by  $-.58$  units through cortisol's effect on peer acceptance. Examination of the pairwise contrasts of the indirect effects revealed that the magnitude of the specific indirect effect through peer acceptance was significantly larger than the indirect effect through teacher closeness (BCa 95% CI =  $.15$  to  $1.54$ ).

For proactive aggression, the results were also consistent with findings from the simple mediation approach and Sobel test. Examination of the specific indirect effects indicated that teacher closeness was a unique mediator of the association between afternoon cortisol and teacher-rated proactive aggression. In contrast to the findings using the causal steps and Sobel test approach, peer acceptance was a unique mediator of teacher-rated proactive behaviour. The results suggest that both mediators contributed to the indirect effect above and beyond the presence of the other mediator, age, and gender. As afternoon cortisol levels increased by one unit, teacher reported proactive aggression decreased by  $.05$  units through cortisol's effect on teacher closeness; and by  $.04$  units for peer acceptance- controlling for peer acceptance, age and gender.

For social aggression, the results were consistent with findings from the simple mediation model but not the Sobel test. The findings suggest that peer acceptance is a unique mediator of the association between afternoon cortisol and teacher-rated social aggression. As afternoon cortisol levels increased by one unit, teacher reported social aggression decreased by  $.22$  units through cortisol's effect on peer acceptance, controlling for teacher closeness, age and gender.

In summary, the results suggest that afternoon cortisol is significantly and inversely associated with specific subtypes of aggressive behaviour, and positively associated with prosocial behaviour in a cross-sectional sample of children in middle childhood. Furthermore, results from the multiple mediation analyses, provide some initial support for the hypothesis that peer acceptance and teacher closeness uniquely mediate the association between afternoon cortisol to peer- and teacher-reported behaviours. However, given the cross-sectional nature of the study and lack of temporal precedence of variables for mediation analyses, the alternative model with behaviour as the independent variable and HPA axis as the dependent variable was addressed in a series of exploratory analyses. The following section presents preliminary findings exploring the alternative hypothesis that children's behaviour predicts afternoon cortisol in a classroom setting. The purpose of this exploratory analysis is to illustrate the importance of including social-contextual variables, such as supportive relationships with peers and teachers, as mediating variables in biological studies of behavioural development and to highlight a limitation of cross-sectional research studies.

### **Exploratory Analyses**

This was a cross-sectional study that sampled daily salivary cortisol in a classroom setting within 2 weeks of measuring peer- and teacher-reported behaviours and supportive relationships. The study was based on the hypotheses that children's daily cortisol obtained over four consecutive days is an index of children's everyday HPA axis activity in a naturalistic context, and that daily HPA axis activity is significantly associated with children's typical everyday behaviours rated at the end of a school year by peers and teachers. However, one of the requirements of mediation analyses is temporal precedence, where the predictor variable cortisol must temporally precede the mediators, which in turn must precede the dependent variable, behaviour. This cross-sectional study did not adequately capture temporal precedence of

variables and so it is possible that children's behaviour precedes or predicts afternoon cortisol. Therefore, to address this alternative model, the individual multiple mediation models were repeated with each individual teacher- and peer-rated behaviour as predictor variables and each index of cortisol as the dependent variable (i.e., morning, noon, afternoon cortisol, diurnal cortisol slope and AUC). Age and gender were included as covariates. Findings revealed a significant mediating influence of teacher closeness on the association between peer-nominated prosocial behaviour and afternoon cortisol, and teacher rated proactive aggression and afternoon cortisol (see Appendix D, Table D19). The positive point estimate for peer nominated prosocial behaviour is consistent with the interpretation that higher ratings of peer prosocial behaviour are positively associated with teacher closeness, which in turn, is positively associated with higher afternoon cortisol. The negative point estimate for teacher-rated proactive aggression suggests that higher ratings of teacher-rated proactive aggression was associated with lower teacher closeness, which was associated with lower afternoon cortisol. Peer acceptance was shown to influence the association between teacher-rated social aggression and afternoon cortisol. Specifically, higher ratings of teacher-reported social aggression were associated with both lower peer acceptance, and subsequent lower afternoon cortisol. Including children's time of awakening or the effects of belonging to one of the four classrooms did not influence the significance of the results. These preliminary findings illustrate that both teacher closeness and peer acceptance exert a mediating influence on the association between cortisol and specific subtypes of behaviour irrespective of the directionality of the model. These results highlight the potential importance of including social-contextual variables, such as supportive relationships with peers and teachers, as mediating variables in biological studies of behavioural development.

## CHAPTER 4

### Discussion

In a recent special issue addressing the importance of biological measures in developmental research, Cicchetti and Gunnar (2007) emphasized the importance of examining causal models of behavioural development to elucidate the interplay among various risk factors at multiple levels of biological and psychological functioning (e.g., Cicchetti & Curtis, 2007). Likewise, prominent investigators in developmental research have called for an emphasis on protective factors that facilitate positive adaptation such as nurturing and supportive relationships with others (see Luthar, 2006; Luthar & Brown, 2007; Masten, 2001). In response to these converging recommendations, the current study was designed to identify processes associated with prosocial and aggressive behaviours in school-age children in classroom contexts. The purpose of this study was to examine the associations of daily patterns of salivary cortisol to peer- and teacher-rated reactive, proactive, and social aggression and prosocial behaviours. Additionally, this study investigated the unique mediating influence of peer acceptance and teacher closeness on the association between daily salivary cortisol patterns and behaviour. Several key findings emerged. First, the findings suggest that multiple samples of salivary cortisol obtained in a naturalistic classroom setting are significantly associated with peer- and teacher-ratings of prosocial and aggressive behaviours. Specifically, the findings revealed that lower afternoon cortisol significantly predicted higher levels of proactive, reactive, and social aggression. Secondly, a significant positive association was observed between afternoon cortisol and prosocial behaviour. Thirdly, children's supportive relationships with their peers and teachers were shown to influence the association between afternoon cortisol and prosocial and aggressive behaviours. Specifically, higher afternoon cortisol predicted higher levels of prosocial behaviour via higher levels of peer acceptance and teacher closeness, whereas lower afternoon cortisol was found to predict higher rates of aggressive behaviours via lower

levels of peer acceptance and teacher closeness. Exploratory analyses examining the alternative model with cortisol as the dependent variable provided further support for the mediating influence of peer and teacher supportive relationships on the association between daily cortisol and behaviour. Taken together, the findings from this cross-sectional research provide preliminary evidence for the importance of including relational-contextual variables, such as peer and teacher relationships, in studies of children's behavioural development and hence may suggest new avenues for classroom-based research and practical intervention.

The findings will be discussed in the following order. First, the association between daily patterns of cortisol and behaviour in a classroom setting will be discussed followed by a discussion of the mediating influence of peer acceptance and teacher closeness. Next, the association between daily patterns of cortisol to: (1) proactive and reactive aggression, (2) social aggression, and (3) prosocial behaviour will be discussed as well as the mediating influence of peer acceptance and teacher closeness. Finally, limitations and directions for future research are proposed.

### **Associations of Daily Patterns of Salivary Cortisol to Behaviour in a Classroom Setting**

The goal of this research was to examine children's everyday salivary cortisol patterns in relation to their behaviours and social relationships in an everyday classroom context. To achieve this goal, the study was designed to capture peer- and teacher-reports of children's social behaviours at the end of an academic school year when children and teachers were familiar with each other. Within two weeks of measuring behaviours, multiple samples of cortisol were obtained during a typical school week to capture children's typical daily HPA axis activity within their classroom environment at that particular time of year. The results suggest that low afternoon cortisol measured in a classroom context is associated with higher ratings of proactive, reactive, and social aggression, and lower ratings of prosocial behaviour in school-age children. These findings are consistent with the view that daily patterns of salivary cortisol provide

valuable information about the role of the HPA axis in predicting children's behaviour in middle childhood (see Adam & Kumari, 2009; Alink et al., 2008; Matthews, 2002; Weinstock, 2008). The results are also consistent with previous research observing an inverse association between low daily cortisol and externalizing behaviour problems in children (see Alink et al., 2008 for a review; e.g., Loney et al., 2006; McBurnett et al., 2000; Oosterlaan et al., 2005; Pajer et al., 2001; Shirtcliff et al., 2005; Shoal et al., 2003). A common explanation for the association between low cortisol and aggressive behaviours is attributed to the proposal that cortisol mediates an inhibited, fearful, or anxious state. Thus, low levels of cortisol may signal biological under arousal contributing to stimulation-seeking or fearlessness, which in turn makes aggression more likely (e.g., Raine, 1996; van Goozen et al., 2000). Moreover, those children who often seek stimulation may be involved in frequent stressful situations and eventually habituate to these stimuli and show a blunted stress response or low basal cortisol levels (Alink et al., 2008; van Goozen et al., 2007). In effect, behaviours such as aggression may provide under-aroused children with stimulation that increases their cortisol levels to more comfortable levels (Murray-Close et al., 2008). Fearlessness has also been associated with low cortisol and aggression due to a lack of inhibition of being involved in aggressive behaviours (Raine, 2002). What is not clear from the results of the current study is why afternoon cortisol and not other indicators of daily patterns of cortisol, such as morning levels, diurnal slope or total cortisol output (AUC), were significantly associated with children's behaviour. The results are difficult to interpret given the existing research suggesting that different indices of daily patterns in cortisol, specifically those measures that tap into morning or afternoon aspects of the diurnal rhythm, may differentially index psychosocial stressors (e.g., socioeconomic background) in different environmental contexts (e.g., workplace, home). Nonetheless, the current findings will be discussed in reference to some of the extant empirical research exploring different indices of

daily cortisol (e.g., morning, afternoon) in various populations (e.g., children, adults) across diverse settings (e.g., school, home, and work).

At the time of this review, limited research exists examining daily patterns of cortisol in school-age children in classroom settings in relation to behaviour. Of the few investigations sampling salivary cortisol from school-age children in classrooms some researchers have focused on the effects of socioeconomic status as a measure of early chronic stress. The findings are equivocal, suggesting that children from low SES backgrounds demonstrate higher morning and evening cortisol and elevated AUC cortisol (e.g., Gustafsson et al., 2006; Lupien et al., 2001), or no relationship between SES and one sample of morning cortisol sample in a large sample of adolescents (West et al., 2010). For example, Gustafsson et al. (2006) sampled salivary cortisol three times a day (i.e., morning, late morning, and evening) over three days in a school setting from a sample of typically developing Swedish children (aged 6 to 12 years). The authors observed higher morning cortisol in children from low SES families and children with immigrant parents and higher evening cortisol and greater total daily output of cortisol (AUC) in children with psychiatric symptoms. Similarly, research by Lupien et al. (2001) found that children from lower SES backgrounds demonstrate higher morning cortisol in a school setting. Parallel research in a home setting suggests that children from lower SES households demonstrate increasing daily cortisol output (area under the curve), but no change in diurnal cortisol slope in a home setting over two years (Chen, Cohen, & Miller, 2010). Thus, it could be predicted based on these findings that higher morning cortisol sampled in a classroom context may be significantly associated with increased ratings of peer- and teacher-reported aggressive behaviours. However, the current study found that children's lower afternoon cortisol was more strongly associated with peer- and teacher-ratings of aggressive and prosocial behaviour than morning cortisol or change in cortisol across the day (i.e., diurnal slope). Adequate comparisons cannot be made among these studies as children's SES status was not measured in the current

study and afternoon cortisol was not sampled in the existing literature on classroom-based research described above (e.g., Gustafsson et al., 2006; Lupien et al., 2001; West et al., 2010). What may be more relevant to studies of salivary cortisol in developmental research is the concept that different indicators of daily patterns in cortisol, such as high morning or evening cortisol, are associated with specific psychosocial stressors, such as SES status or psychiatric symptoms, respectively (Gustafsson et al., 2006). Indeed, parallel research examining chronic stress in adults suggests that different aspects of job stress differentially influence the cortisol awakening response compared to cortisol sampled across the day (Kunz-Ebrecht et al., 2004b; Steptoe, Cropley, Griffith, & Kirschbaum, 2000). These findings may help explain why afternoon cortisol is associated with behaviours in a classroom setting, while chronic stress is associated with higher morning cortisol in children and adults across diverse settings. To explore this concept further, the following discussion introduces two investigations in adults showing that higher morning cortisol is a predictor of long-term and concurrent workplace stress. For example, multiple samples of salivary cortisol were obtained from schoolteachers during a typical workday in a school setting (e.g., Steptoe et al., 2000). Higher morning cortisol was observed in schoolteachers who had reported higher job strain and greater expressions of anger 12 months earlier. No differences in cortisol were observed during later parts of the day such as the afternoon or evening. The authors attributed the higher morning cortisol to anticipation of a stressful workday. Notably, these results were constrained by the lack of control for individuals' time of awakening or morning activities. In a more recent study, the investigators examined whether variations in subjective reports of work stress in middle-aged adults accounted for differences in the cortisol awakening response and average cortisol concentrations (average of eight cortisol samples from morning to evening) obtained across a single working day (Kunz-Ebrecht et al., 2004b). Their findings suggest that the cortisol awakening response was positively associated with high job demand, which was attenuated by higher SES. In women, but not men,

the average value of multiple cortisol samples obtained over the course of the work day were higher in lower SES women with high job demands, but lower in women from low SES backgrounds with low job demands. These cortisol differences were independent of age, and time of awakening (Kunz-Ebrecht et al., 2004b). Taken together, these investigations in adults and the workplace as well as the few studies examining chronic stress and cortisol in classroom settings, suggest that different indices of daily patterns in cortisol, specifically those measures that tap into morning or afternoon aspects of the diurnal rhythm, may offer different information. Some researchers claim that sampling salivary cortisol in the afternoon is preferable to morning as it reflects a relatively quiescent period of the circadian release of cortisol compared with morning hours (Jessop & Turner-Cobb, 2008) and consequently minimizes the extent of within-person variability (Smider et al., 2002). Furthermore, research suggests that morning and afternoon cortisol offer information about intrinsic biological processes (i.e., diurnal rhythms) and extrinsic contextual processes such as social and emotional events, respectively (see Shirtcliff & Essex, 2008). Specifically, it is suggested that morning levels are influenced by unique factors that are largely genetic (Bartels et al., 2003), whereas afternoon levels are less under genetic influence and are more easily influenced by the immediate social context (Schreiber et al., 2006). This perspective may be one tentative explanation for why afternoon cortisol in the current study was more closely associated with children's relational context, or peer and teacher supportive relationships, than morning cortisol. In fact, one possible explanation for the variation in findings in research examining daily cortisol in children and adults to psychosocial stressors may be the lack of examination of contextual mediating variables such as supportive relationships with others to specific aspects of the cortisol diurnal rhythm. The findings from the current study suggest that children's supportive relationships with peers and teachers influence the association between afternoon cortisol and behaviour in a classroom setting. The following discussion situates these findings in reference to some of the

existing research in young children attending day care, where it is suggested that young children's daily cortisol patterns are associated with peer social interactions and the quality of relationships with day care providers.

### **Mediating Influence of Peer Acceptance and Teacher Closeness**

Previous research examining daily patterns of cortisol in children residing in everyday social contexts has focused on younger children attending day care (see Gunnar & Quevedo, 2007; see Vermeer & van IJzendoorn, 2006 for a review and meta-analysis). Findings show that there tends to be a secondary increase in cortisol in the afternoon among young children attending full-day centre-based care compared to home-based day care (e.g., Watamura et al., 2003). This increase in cortisol has been attributed to the emerging salience of peer social relationships at this age and young children's inability to cope with social interactions (see Gunnar & Quevedo, 2007). It is thought that young children have immature social and coping skills likely leading to an inability to manage the demands of peer interactions. Combined with long hours at day-care, the young child's ability to regulate cortisol is thought to be taxed resulting in an increase in morning to afternoon cortisol (see Gunnar & Quevedo, 2007; Watamura et al., 2003). These findings suggest that higher afternoon cortisol in these younger children is an indicator of children being under stress. In contrast, the results of the current study suggest that in older school-age children, higher afternoon cortisol sampled in classroom contexts is associated with positive behaviour and supportive relationships with peers and teachers. One possible explanation for these findings is that daily patterns in cortisol in a peer-based setting such as day care or classrooms are on a developmental continuum; where high afternoon cortisol is an indicator of stress in young children, but an indicator of positive development by middle childhood. However, this remains to be determined. Nonetheless, evidence from research on chronic stress in early childhood suggests that over time, stressful environments may result in downregulation of HPA axis activity (see Gunnar & Quevedo, 2007;

Tarullo & Gunnar, 2006). It is possible that HPA dysregulation leads to lower afternoon cortisol levels by middle childhood that are a risk factor for decreased quality of social relationships and an increased propensity for aggression (e.g., Alink et al., 2008; Matthews, 2002; Weinstock, 2008). In fact, the importance of children's social relationships on the developing HPA axis is found in emerging research showing that the age-related increase in cortisol in young children is not observed in high quality home-based day care contexts with individualized, supportive relationships with day care providers (e.g., Dettling et al., 2000; Ouellet-Morin et al., 2010; Watamura, Coe, Laudenslager, & Robertson, in press; Watamura et al., 2003; Watamura et al., 2009). Sensitive, responsive caregiving by a primary caregiver is thought to promote secure attachment in toddlers, thus ameliorating the observed elevations in cortisol observed in the absence of a caregiver in day care centres (see Tarullo & Gunnar, 2006). The findings from the current study parallel those observed with younger children in high quality day care.

Specifically, the results suggest that a close, supportive relationship with a teacher explains in part the association between high afternoon cortisol and increased ratings of prosocial behaviour. Given the importance of peers in middle childhood, the results are also consistent with a model where higher ratings of peer acceptance is a process by which higher afternoon cortisol predicts prosocial behaviours. Supportive relationships with peers and teachers function as a resource during middle childhood when children are faced with challenges or difficulties, permitting them to respond with more vigour, flexibility, and constructive and prosocial actions (Furrer & Skinner, 2003). Thus, children with higher afternoon cortisol may be better equipped to interact socially with peers and teachers leading to an increased propensity for prosocial behaviours and decreased aggression.

### **Distinct Mediating Influence of Peer Acceptance and Teacher Closeness**

A key observation in the current study was the finding that the mediating influence of peer acceptance was distinct from the influence of teacher closeness. These results are consistent

with theory suggesting that teachers are important to children for different reasons than peers (Darling & Hamilton, 2003). Moreover, these results suggest that having a balance of peer and adult support has both an additive and positive influence on children's behavioural development (e.g., Buchanan & Bowen, 2008; Laible, Carlo, & Raffaelli, 2000). Further research is needed to understand the processes by which peer acceptance and teacher closeness uniquely influence the association between daily patterns in cortisol and behaviours. Middle childhood represents an important developmental period when children start to form strong attachments to friends and value the intimacy and reciprocity in these relationships (see Pedersen, Vitaro, Barker, & Borge, 2007). Evidence suggests that peer relationships provide unique opportunities for children to learn and practice prosocial skills in an egalitarian and reciprocal manner (Piaget, 1965; Wentzel & McNamara, 1999). Hence prosocial, sharing, and helpful behaviours develop out of peer interactions that adopt principals of reciprocity and mutual respect such as peer acceptance (Hartup, 1992; Kohlberg & Kramer, 1969). In contrast, children's desire to obtain status in peer hierarchies may motivate children to engage in specific forms of aggression such as social aggression (Neal, 2010). The current results suggest that lower afternoon cortisol is associated with lower acceptance by peers and increased propensity to engage in aggression. Lower afternoon cortisol in middle childhood may be a result of down regulation of the HPA axis in response to the chronic stress of unstable social systems such as peer hierarchies across early childhood (Sapolsky, 2005). Dominance hierarchies occur in many species including humans and the stress associated with social rank is shown to influence stress physiological systems such as the HPA axis (Sapolsky, 2005). For example, preliminary research by West and colleagues (2010) suggests that adolescents' self-rated position on school-based social hierarchies (e.g., peer, sport, scholastic) is associated with a single sample of their morning cortisol obtained in a school setting. Adolescents' who rated themselves lower on peer-, scholastic-, and sport-based hierarchies demonstrated higher morning cortisol in one sample obtained in a classroom setting.

The authors found no association between morning cortisol and SES status, which is inconsistent with previous research showing an association between higher morning cortisol and chronic stress (such as lower SES status) in children in middle childhood (e.g., Gustaffson et al., 2006; Lupien et al., 2001) and adults (Steptoe et al., 2000). West et al. (2010) interpret their findings to suggest that school social hierarchies are more important than SES in this sample of adolescents, aged 15 years. Notably, the results of the West et al. (2010) study suggest that females who rated themselves at the top of the social hierarchy demonstrate higher morning cortisol, which is consistent with the perspective that position within social hierarchies is stressful both at the bottom and the top of the hierarchy during middle childhood and adolescence (Neal, 2010; Oldehinkel et al., 2007). The higher morning cortisol observed in the study by West et al. (2010) could be seen to contradict the findings from the study, however, it also possible that high morning or low afternoon are both accurate representations of HPA axis dysregulation. Notably, the research by West et al. (2010) was limited by the lack of multiple sampling of cortisol across the school day. The single sample of morning cortisol was obtained after the beginning of administration of questionnaires, which may have resulted in uncharacteristically high cortisol values. Clearly, the research in this area is still in its infancy and further research is needed to tease out the associations among daily patterns of cortisol and indices of behaviour and peer relationships in classroom contexts. Given the separate unique mediating influence of teacher closeness, an interesting avenue to pursue is the possibility that supportive relationships with teachers may disrupt this negative cycle of HPA axis dysregulation, low peer acceptance, and aggression. Teachers are thought to represent a middle ground between parents and peers; representing adult expectations, but are less responsible for discipline compared to parents, thus fostering different degrees of communication and closeness (Darling & Hamilton, 2003). Children who are rated by their teachers as having a close, supportive student-teacher relationship likely feel increased levels of school belonging which is a significant predictor of

psychological and behavioural functioning in middle childhood (see Baker, 2006; Baker, Grant, & Morlock, 2009; Danielsen, Samdal, Hetland, & Wold, 2009; Roeser, Midgley, & Urdan, 1996). In fact, the findings from the exploratory analyses provide preliminary support of the positive influence of supportive relationships with peers and teachers on the association between afternoon cortisol and behaviour, regardless of the direction of association between cortisol and behaviour. However, these results should be interpreted with caution given the cross-sectional nature of the study and the lack of temporal precedence of the variables. As such, it is possible that daily patterns in salivary cortisol are a consequence and/or a cause of children's behaviour (see Alink et al., 2008; Gunnar & Vazquez, 2001; Sondejiker et al., 2008). Ecological transactional models of child development suggest that there are multiple levels of children's ecologies (i.e., internal physiological, external relational context) that mutually influence each other and children's behavioural adaptation over time (see Cicchetti & Lynch, 1993; Lynch & Cicchetti, 1998). It is therefore possible that involvement in prosocial or aggressive behaviours leads to changes in neurobiological functioning and levels of supportive relationships with peers and teachers. Similarly, different levels of peer acceptance and teacher closeness may differentially influence the degree to which students engage in prosocial or aggressive behaviours that directly or indirectly influence daily HPA axis activity (see Gunnar & Vazquez, 2001). Despite the limitations of cross-sectional research, the current study was situated in theoretical and empirical evidence justifying the position of prosocial and aggressive behaviour as dependent variables. Importantly, the current study was based on previous work suggesting that early experience is associated with specific patterns in daily cortisol by middle childhood (Cicchetti et al., 2010; El Sheik et al., 2008; Murray-Close et al., 2008). The theoretical basis of this research was founded on the perspective that multiple measurements of basal cortisol in a classroom context represent children's typical everyday HPA axis activity, and that daily HPA axis activity is a predictor of children's behaviours measured near the end of the school year.

Consistent with previous work of this nature, daily patterns in salivary cortisol were deemed as significant predictors of children's behaviours rated by peers and teachers near the end of a school year (e.g., El Sheik et al., 2008; Murray-Close et al., 2008). As such, the results of this research on prosocial and aggressive subtypes of behaviour will continue to be discussed within the model of behaviour as the dependent variable. Limitations to this model are discussed in a later section.

The following section will move towards the discussion of findings linking daily patterns of salivary cortisol to: (1) proactive and reactive aggression, (2) social aggression, and (3) prosocial behaviour, and the mediating influence of peer acceptance and teacher closeness on each of these behaviours. The results will be discussed in relation to previous research exploring daily cortisol patterns across diverse populations in different contexts.

### **Daily Patterns of Salivary Cortisol and Reactive and Proactive Aggression**

One of the goals of this research was to examine the association between daily HPA axis activity, indexed by daily patterns of salivary cortisol, and proactive and reactive subtypes of aggression. Reactive aggression is defensive and retaliatory aggression and is characterized as a highly aroused aggressive response to a real or perceived provocation. Proactive aggression is defined as planned, goal-directed, low-arousal behaviour focused on an anticipated goal such as material possession or social dominance (Dodge, 1991; Dodge & Coie, 1987; Kempes et al., 2005). This study was based on the premise that measurement of daily patterns of salivary cortisol offers a window into the associations between children's daily HPA axis activity and behaviours in an everyday classroom context. Based on prior research suggesting that low basal cortisol is significantly associated with both reactive and proactive aggressive behaviours (Poustka et al., 2010), it was predicted that low daily cortisol would be associated with higher ratings of reactive aggression and proactive aggression. This hypothesis was specific to measurement of daily patterns of salivary cortisol obtained in a naturalistic setting (i.e., daily

HPA axis activity) which differs from that which would be expected under conditions of acute stress, where frustration, anger and high post-stressor levels of cortisol (i.e., reactivity of the HPA axis) are associated with reactive aggression, but not proactive aggression (e.g., Hubbard et al., 2002). Consistent with predictions, lower afternoon cortisol was associated with higher ratings of peer- and teacher-reported reactive aggressive behaviours, and higher ratings of peer-reported proactive aggression.

The observation that lower afternoon cortisol was associated with increased ratings of proactive and reactive aggression is consistent with emerging empirical research in the field of cortisol and externalizing behaviour in youth (see Alink, 2008 for a meta-analysis). However, this finding is not directly consistent with research by Murray-Close and colleagues (2008) where it was found that children rated as physically aggressive demonstrated higher levels of cortisol following morning arrival at a summer day camp and a steeper decline in cortisol over the day (i.e., lower afternoon cortisol). Murray-Close and colleagues did not measure specific subtypes of aggression but suggest that the observed steep decline in daily cortisol reflected HPA axis dysregulation in a subset of physically aggressive children, specifically reactive aggressive children. A common explanation for the association between steeper decline in diurnal cortisol and aggression is that low cortisol represents decreased fear and under-arousal and a propensity for stimulation seeking via increased aggressive behaviours. The current findings expand on previous research to suggest that low afternoon cortisol is associated with specific subtypes of reactive and proactive aggression in school-age children in classroom contexts (e.g., Murray-Close et al., 2008; Poustka et al., 2010). A potential explanation for the significant association between low afternoon cortisol in a classroom context and higher rates of proactive and reactive aggression may be attributed to measurement of daily, basal HPA axis activity rather than stress reactivity of the HPA axis. It is possible that under basal, everyday conditions in the absence of an acute social stressor, the neurobiological correlates of proactive and reactive aggression are

indistinguishable. For example, laboratory-based studies that index HPA axis stress reactivity are tapping into the child's ability to mount a stress response to an acute stressor (e.g., public speaking task). As such, HPA axis stress reactivity may be more strongly associated with an outburst of reactive aggression compared to proactive aggression under conditions of acute stress. It is therefore possible that distinct neurobiological profiles for proactive and reactive aggression are more readily defined in studies examining cortisol in response to an acute social challenge (i.e., HPA axis reactivity), rather than daily or basal activity of the HPA axis. In other words, daily HPA activity and acute HPA stress reactivity may differentially index subtypes of aggressive behaviour. This explanation is supported by empirical research showing that reactive but not proactive aggression is associated with HPA axis reactivity in response to a laboratory induced social stressor (e.g., Clanton, 2007; Hubbard et al., 2002; Lopez-Duran, et al., 2009). Whereas under resting conditions in a laboratory setting, lower afternoon cortisol (5pm) is significantly and inversely associated with parent-reported proactive and reactive aggression in a large sample of high-risk adolescent males (e.g., Poustka et al., 2010). The methodological design of the Poustka et al. study was limited by the use of only one blood plasma cortisol sample obtained by venipuncture (i.e., needle) in a laboratory setting. The results of the current study extend these findings to suggest that afternoon cortisol is associated with peer- and teacher-reported reactive aggression and peer-nominated proactive aggression in a non-clinical sample of children in an everyday classroom context.

Another possible explanation for the observation that both peer-nominated proactive and reactive aggression were significantly and inversely associated with afternoon cortisol may be that there was high overlap between these two subtypes of aggressive behaviour. The strong positive correlation between proactive and reactive aggression obtained in the current investigation ( $r = .60$  to  $.69$ ) suggests that the children may have been exhibiting both subtypes of aggression. Evidence suggests that proactive and reactive aggression co-occur with children

displaying both subtypes of aggression (see Hubbard et al. 2010). In fact, only small subgroups have been characterized as reactive-only or proactive-only in middle childhood (Kempes et al, 2005). The controversy surrounding the overlap between proactive and reactive aggression has spurred a recent debate into the measurement of these subtypes of aggressive behaviours. In a recent meta-analysis of proactive and reactive aggression in childhood and adolescence and the differential relations with psychosocial adjustment, Card and Little (2006) recommend that future research into the correlates of proactive and reactive aggression will likely benefit from measures that provide distinct assessments of the functions of these subtypes (see Card & Little, 2006 for a review). Accordingly, in their recent review of precursors, correlates, and measurement of proactive and reactive aggression in children and adolescents, Hubbard and colleagues (2010) advocate the use of questionnaires that are worded to emphasize reactive and proactive behaviour rather than the emotion anger. Anger and anger regulation are critical components of reactive aggression, whereas proactive aggression is goal motivated, and potentially unemotional (see Hubbard et al., 2010). Thus, the questionnaires employed in the current study, originally designed by Dodge and Coie (1987) that specifically index reactive behaviours according to anger may not be useful for assessing neurobiological correlates of aggression. What may be more relevant for future studies examining proactive and reactive aggression are questionnaires that tap into the motivations underlying children's behaviour rather than the experience of anger (see Hubbard et al., 2010).

### **Mediating Influence of Peer and Teacher Supportive Relationships on Daily Patterns of Salivary Cortisol and Reactive and Proactive Aggression**

One of the unique contributions of this study was the inclusion of intermediating relational variables in the examination of the neurobiological correlates of proactive and reactive sub-types of aggression. The hypothesis that children's supportive relationships with peers and teachers act as explanatory process variables in the association between daily patterns in salivary

cortisol and proactive and reactive aggression was partially supported by this study. The findings revealed a significant mediating influence of peer acceptance and teacher closeness on the association between afternoon cortisol and teacher-rated, but not peer-rated, proactive aggression. Specifically, lower levels of afternoon cortisol predicted higher levels of teacher-rated proactive aggression via lower levels of peer acceptance and teacher closeness. The findings also revealed that lower afternoon cortisol predicted higher levels of teacher-rated reactive aggression via lower levels of peer acceptance.

A potential explanation for the significant findings observed with teacher-rated but not peer-rated proactive and reactive aggression may be that teachers with advanced socio-cognitive abilities are more capable of accurately assessing children's motivations for either proactive or reactive aggressive behaviour. Children may be aware of a peer demonstrating general aggressive behaviour but may not be developmentally mature enough to determine the specific motivation behind the behaviour and thus unable to rate the sub-type of aggression. Alternatively, it may be that children can accurately assess proactive and reactive aggressive behaviours in their peers, but utilize different criteria for assessing these behaviours that were not captured by our measure. It is also possible that the questionnaire items used to assess these behaviours were conceptually distinct for teachers compared to peers. These explanations highlight the importance of obtaining ratings of behaviour from multiple sources and emphasize the need for more specific measures of aggression that tap into children's behavioural motivations rather than subjective expressions of anger. The mediating influence of supportive relationships with peers and teachers will be discussed for reactive and proactive separately below.

**Reactive aggression.** Consistent with our predictions, lower ratings of peer acceptance were shown to mediate the association between low afternoon cortisol and higher levels of teacher-rated reactive aggression. In contrast to expectations, no mediating influence of teacher

closeness was observed. In fact, results of the bootstrapping contrasts between the two indirect effects revealed that the magnitude of the indirect effect of peer acceptance was significantly greater than that observed for teacher closeness. These results suggest that lower ratings of peer acceptance may play a key role in explaining the inverse association between afternoon cortisol and reactive aggression in middle childhood. This finding is supported by evidence showing that reactive aggression more so than proactive aggression is related to peer relationships (see Card & Little, 2006). Most existing research however has tended to focus on the association between peer rejection and reactive aggression (e.g., Boivin, Dodge, & Coie, 1995; Dodge et al., 2003; Ladd & Burgess, 2001; Morrow, Hubbard, McAuliffe, Rubin, & Dearing, 2006; Poulin & Boivin, 2000). These investigations are based on the premise that peer rejection and victimization may promote situations in which a child reacts aggressively to peer provocations as well as promote beliefs that peer's intentions are hostile (e.g., Card & Little, 2006; Crick & Dodge, 1996; Dodge & Coie, 1987; Kempes, Matthys, Maassen, van Goozen, & van Engeland, 2006). Furthermore, evidence from neurobiological studies of aggression suggest that altered HPA axis activity may represent dysregulation of underlying neural circuitry in the frontal cortex associated with basic response to threat (see Blair, Karnik, Coccaro, & Steiner, 2010). Research suggests that children with altered stress response circuitry are more likely to attribute ambiguous situations as hostile (Locke et al. 2009), possibly leading to greater peer rejection or lower peer acceptance and reactive aggression. The propensity for children to engage in reactively aggressive behaviours may in turn lower peer acceptance leading to chronic stress and dysregulation of daily HPA axis activity. These cyclical associations may unfold repeatedly over short or long periods during early childhood so that by middle childhood, altered baseline activity in basic threat circuitry, low salivary cortisol (i.e., low daily HPA axis activity), and chronic low peer acceptance are evident. For this reason, longitudinal research is needed to tease

apart the biological and relational correlates of behavioural development across childhood and adolescence.

**Proactive aggression.** Consistent with the study predictions, lower ratings of peer acceptance and teacher closeness uniquely mediated the association between low afternoon cortisol and higher levels of teacher-rated proactive aggression in typical everyday classrooms. This finding was perhaps surprising given that no significant direct association was observed between afternoon cortisol and proactive aggression (see path *c*; Figure 1 and 2). However, strong associations were observed for the indirect path via the mediators (paths *a* and *b*), suggesting that the indirect path via peer acceptance and teacher closeness accounted for more variation between afternoon cortisol and proactive aggression than the direct association (path *c*). In fact, the indirect paths (path *a* and path *b*) between afternoon cortisol, supportive relationships, and behaviour were statistically significant supporting a mediating effect using the Sobel test and bootstrapping approaches. These findings underscore the recommendation not to rely on statistical significance of direct causal paths when examining models of mediation (Mackinnon et al., 2002).

One possible explanation for the mediating influence of peer acceptance and teacher closeness on the association between low afternoon cortisol and higher levels of teacher-rated proactive aggression can be found in studies examining the socio-cognitive processes that underlie the motivation to engage in proactive aggression. Current theoretical perspectives suggest that proactive aggression is goal-oriented behaviour characterized by low daily biological arousal (e.g., Hubbard et al., 2002). It is therefore possible that children with low afternoon cortisol are chronically under-aroused demonstrating sensation-seeking and fearlessness that results in negative social interactions (Raine, 2002) and lower peer acceptance and teacher closeness. The lower levels of arousal and lower quality relationships with peers and teachers possibly leads to an increased propensity to engage in proactive aggressive behaviours

that have the goal of material gain or social dominance (Dodge & Coie, 1987; Hubbard et al., 2010). Engaging in proactive aggression likely decreases peer acceptance and teacher closeness that may lead to a chronic feedback cycle that perpetuates physiological under-arousal and the drive to engage in proactively aggressive behaviours.

These findings are some of the first to suggest that both peer acceptance and teacher closeness provide unique individual influences in the inverse association between afternoon cortisol and proactive aggressive behaviours in middle childhood. Previous longitudinal research in adolescent males has shown that parental supervision and parental substance abuse moderates the association between proactive aggression and delinquency in later adolescence and adulthood (Brendgen et al., 2001). Findings from the present study expand upon this knowledge to suggest that non-familial classroom supportive relationships are also important mediating influences in the prediction of proactive forms of aggression. The evidence that environmental factors may differentially predict proactive and reactive aggression is supported by evidence from a recent study with 6-year-old twin pairs, suggesting that reactive and proactive aggression may be influenced mostly by socialization experiences specific to each type of aggression and only to a small degree by genetic effects (Brendgen, Vitaro, Boivin, Dionne, & Perusse, 2006). The findings from the current study have important implications for the design of classroom-based prevention and intervention efforts aimed to enhance teacher and peer relationships. Given the unique mediating influence of both peers and teachers, it is possible that peer acceptance and teacher closeness represent two different pathways to ameliorate classroom behavioural maladaptation. Furthermore, in the absence of supportive relationships with peers, supportive, close relationships with teachers may buffer children at risk for engaging in proactively aggressive behaviours. Further research is needed to disentangle the mechanisms by which supportive relationships with peers and teachers influence bio-behavioural adaptation. What is clear from this research is that supportive relationships with peers and teachers should be taken

into account in future studies aimed at understanding the associations between daily patterns in salivary cortisol and social behaviours in typical everyday classroom contexts.

### **Daily Patterns of Salivary Cortisol and Social Aggression**

Another goal of this research was to investigate the association between daily patterns in salivary cortisol, and peer- and teacher-reported social aggression in a typical everyday classroom environment. Social aggression is defined as behaviours such as gossiping, rumour-spreading and social exclusion that are intended to harm another through damaging relationships (Cairns et al., 1989; Galen & Underwood, 1997). Based on previous research (Murray-Close et al., 2008), it was predicted that low salivary cortisol would be associated with higher levels of social aggression. Consistent with these predictions, a significant inverse relationship between afternoon cortisol and peer-reported social aggression was found, but no significant direct association was observed between afternoon cortisol and teacher-rated social aggression. However, inclusion of peer acceptance as an explanatory mediating variable indicated a significant association between lower afternoon cortisol and greater teacher-rated social aggression via decreased levels of peer acceptance. These findings are some of the first to suggest that peer acceptance may provide an intermediary process by which lower afternoon cortisol is associated with higher teacher- and peer-reported social aggression in everyday classroom contexts.

The observation that lower afternoon cortisol is associated with higher ratings of social aggression in middle childhood is consistent with two current perspectives of aggression. The stimulation-seeking theory of aggression suggests that children with low daily HPA axis activity, or biological under-arousal, engage in social aggression as a stimulating experience that serves to increase physiological arousal to more comfortable levels (Murray-Close et al., 2008). Theories of fearlessness suggest that low cortisol, which inhibits fear, perpetuates a state of fearlessness whereby children are relatively unafraid of the outcomes of their negative behaviour (Raine,

2002). The results of the current study are consistent with one other known study that explored the association between daily patterns in salivary cortisol and social aggression in middle childhood. Murray-Close and colleagues (2008) found an association between higher levels of social aggression and low cortisol in children attending a summer day camp. However, in contrast to the present study, Murray-Close and colleagues found morning cortisol or change in cortisol across the day (i.e., slope) to be a better predictor of social aggression than afternoon cortisol. The difference in findings may be related to the difference in methodology between the two studies. Indeed, the current study was designed to extend previous research assessing daily patterns in cortisol and social aggression in middle childhood in three important ways. First, the current study was designed to measure children's daily patterns in cortisol in an everyday, naturalistic classroom environment. In contrast, Murray-Close and colleagues sampled cortisol in children attending a novel summer day camp where morning cortisol (9am) may have been a reflection of children's neurobiological response to travelling by bus to the summer day camp each morning. Additionally, summer camp is a different environment than a classroom context. Furthermore, the authors acknowledged that they observed a high overlap between their measures of social and physical aggression. As mentioned earlier, it is possible that there are specific aspects of the diurnal cortisol curve that are more relevant to specific forms and functions of aggression depending on the context in which the study takes place. More sophisticated analytical techniques such as growth curve modelling, a technique that permits modelling of change over time, may offer better insight into the specific aspects of the diurnal cortisol rhythm that are associated with social aggression in middle childhood (e.g., Adam, 2006; Adam & Gunnar, 2001; Shirtcliff & Essex, 2008).

A second methodological strength of the current investigation was the examination of daily patterns of salivary cortisol to peer- and, separately teacher- reports of social aggression in a context in which children and teachers were familiar with each other. The study by Murray-

Close et al. (2008) may have been limited by the use of a composite measure of social aggression rated by both camp counsellors and peer nominations. Furthermore, peers and counsellors were perhaps not familiar with children's typical behaviour given that they were in a novel week-long summer camp environment. It is possible that measures of social aggression obtained from peers versus non-familial adults such as camp counsellors or teachers may differ due to measurement issues such as different observations of behaviours and the use of different criteria. Peers compared to teachers have more opportunities to observe socially aggressive behaviours in other contexts outside of the classroom, including the playground and lunchroom. Indeed, in the current study, examination of the direct paths indicated that afternoon cortisol was significantly associated with peer- but not teacher-reports of social aggression. However, once the mediating variable of peer acceptance was included in the model an indirect association between afternoon cortisol and teacher-reported social aggression became evident. A possible explanation for these findings is that the association with afternoon cortisol is direct for peer-nominated social aggression but indirect for teacher-reported social aggression. Why this would be the case is not entirely clear. Social aggression is a peer-based form of aggression; therefore peer ratings of social aggression may more accurately capture those children with daily cortisol patterns that index social aggression. Furthermore, it may be that models of teacher-rated social aggression require a measure of peer socialization (i.e., peer acceptance) before a significant association with cortisol and social aggression is observed. Indeed, developmental research has indicated a decrease in teacher-perceived social aggression between ages 9 to 13 (e.g., Underwood et al. 2009), whereas peer-nominated social aggression is shown to increase in a linear fashion for 9-year-old girls over the course of one calendar year (see Neal, 2010; Murray-Close, Ostrov, & Crick, 2007). Underwood and colleagues (2009) attribute this phenomenon to the increasingly subtle and sophisticated use of social aggression by children and their increasing ability to hide this form of peer-aggression from adults. Thus, investigations that incorporate the use of peer-

nominated ratings of social aggression and peer relational supports may offer deeper insights into the neurobiological correlates of social aggression in typical classroom environments across middle childhood.

### **Mediating Influence of Peer and Teacher Supportive Relationships on Social Aggression**

A third important contribution of the current study was the examination of the mediating influence of peer and teacher relational protective factors on daily patterns in cortisol and social aggression. As mentioned above, the findings suggest that the association between children's afternoon cortisol and teacher-reported social aggression is largely indirect and is mediated in part by other explanatory variables such as peer acceptance. Specifically, children with lower afternoon cortisol and lower ratings of peer acceptance are rated as more socially aggressive by their teachers. A possible explanation for this mediation model may be found through examination of the individual pathways between cortisol to peer acceptance, and peer acceptance to social aggression. For example, current perspectives suggest that low afternoon cortisol or physiological under-arousal may increase the need to engage in negative peer interactions for stimulation thus decreasing ratings of peer acceptance (see Murray-Close et al., 2008). Building on theories of social dominance, low peer acceptance combined with low cortisol or arousal may increase the propensity to engage in socially aggressive behaviours to regain status or popularity, which in turn perpetuates a negative cycle of aggression and low peer acceptance (see Neal, 2010). Efforts to increase peer acceptance may interrupt this cycle of low arousal and social aggression. However, future longitudinal research is needed to address the temporal association among children's daily cortisol patterns, peer and teacher relationships, and social aggression in middle childhood.

### **Daily Patterns of Salivary Cortisol and Prosocial Behaviour**

This study explored the association between daily patterns in salivary cortisol and prosocial behaviours in middle childhood. Results indicated a positive association between

higher afternoon cortisol and higher levels of both peer- and teacher-reported prosocial behaviours. These findings illustrate that afternoon cortisol is not only associated with aggressive behaviours but also with prosocial behaviours in typical classroom environments in middle childhood. Additionally, this research explored the influence of children's peer and teacher supportive relationships as explanatory mediating variables in the association between afternoon cortisol and children's prosocial behaviour. Findings revealed that children with higher afternoon cortisol demonstrated higher levels of peer acceptance and teacher closeness that in turn predicted higher ratings of peer- and teacher-rated prosocial behaviour. These results are consistent with a growing number of studies suggesting that the associations between daily patterns in salivary cortisol (i.e., daily HPA axis activity) and behaviour are influenced by children's social context (e.g., Cicchetti et al., 2010; Murray-Close et al., 2008).

Theoretical support for the positive association between children's afternoon cortisol and supportive relationships with peers and teachers may be found in literature exploring adult 'physiological resourcefulness' in the workplace. In their seminal article on workplace physiology, Heaphy and Dutton (2008) proposed that positive workplace social interactions build individual's physiological resourcefulness; a form of positive health in which the body is able to build, maintain, and repair itself during rest in preparation for challenges during times of stress (Epel, McEwen, & Ickovics, 1998). The authors suggest that positive social interactions build physiological resourcefulness by fortifying the cardiovascular, immune, and neuroendocrine systems that over time contribute to improved physical health (Heaphy & Dutton, 2008). In contrast to traditional models of stress that examine an individual's physiological response to acute or chronic stress, Heaphy and Dutton (2008) suggest that stress is not a necessary prerequisite for physiological resourcefulness but rather physiological resourcefulness can be promoted through social interactions. The notion is that physiological strengthening is obtained through direct exposure to positive social interactions which, in turn

improve an individuals' ability to respond to stress. Translating this perspective to classroom environments, the current findings suggest that children's daily patterns in salivary cortisol may translate into positive social and coping skills that facilitate supportive relationships with peers and teachers and subsequently increase prosocial behaviours. Over time, peer acceptance and teacher closeness may enhance children's social skills and capacity to cope, which translates as inhibitory signals to the stress response system. These inhibitory signals may increase the threshold for activation of the stress response system reducing the ability of challenging stimuli to promote a full stress response from the HPA axis (Bovard, 1959; Seeman & McEwen, 1996). Notably, the directionality of these associations is unclear due to the cross-sectional nature of the present study. What is clear however, is that classrooms are a salient context in which to better understand the influence of peer and teacher supportive relationships on the associations between children's daily cortisol patterns and behaviour.

### **Mediating Influence of Peer and Teacher Supportive Relationships on Prosocial Behaviour**

Findings from the current study revealed that peer acceptance and teacher closeness mediated the significant and positive association between afternoon cortisol and prosocial behaviour. A theoretical explanation for this finding may be found at the intersection of research by Wentzel et al. (2007) on prosocial behaviour and Ladd et al. (1999) on relational styles (i.e., how children seek to establish and maintain relationships with peers and teachers). The findings suggest that children's afternoon cortisol may be an internal physiological motivation to pursue prosocial relational styles that facilitate peer acceptance and teacher closeness that guide children's prosocial behaviour. Incorporating theories of physiological adaptation (McEwen, 1998; Seeman & McEwen, 1996), it is possible that daily HPA axis activity profiles, such as high afternoon cortisol, lead to a higher activation threshold in response to typical everyday stimuli. Thus, children with a higher activation threshold may have better ability to cope with daily stressors and are better able to form positive peer and teacher relationships that

subsequently promote prosocial behaviours. In turn, positive peer and teacher supportive relationships have the potential to feedback as inhibitory signals to the HPA axis maintaining a high threshold for stress activation. What is suggested by these findings is that higher afternoon cortisol may represent positive adaptation of daily HPA axis activity in typical everyday classroom settings. This tentative explanation requires further investigation into the neurobiological correlates of prosocial behaviour in middle childhood, especially given the evidence that young children in high quality day care environments with supportive caregivers do not demonstrate a secondary increase in afternoon cortisol (e.g., Ouellet-Morin et al., 2010; Watamura et al., in press; Watamura et al., 2003; Watamura et al., 2009). The potential beneficial effect of supportive relationships with peers and teachers presents an important avenue for developmental investigators focused on promoting positive adaptation in classroom contexts.

This research builds on existing evidence to suggest that peer acceptance and teacher closeness uniquely mediated the association between afternoon cortisol and prosocial behaviour, even after controlling for the presence of the other mediator, the child's age, and gender. A possible explanation for the distinct mediating effects of peers and teachers may be due to the significant difference in motivations for peer-rated prosocial behaviour compared to teacher-rated prosocial behaviour. The unique influence of peer and teacher supportive relationships is not surprising given that middle childhood is a time of transition in which developmental needs and relational context may conflict. Models of stage-environment fit (e.g., Eccles & Midgley, 1989) highlight children's increasing desire for autonomy while at the same time teachers tend to become more authoritative and maintain more control in the classroom. Thus, children's perceived expectations from peers and teachers may reflect different reasons for engaging in prosocial behaviour. Indeed, research suggests that perceived expectations from teachers are related to external reasons for behaviour such as fear of punishment, whereas perceived expectations from peers to engage in prosocial behaviour are associated with internal reasons

such as feelings of empathy or fear of peer disapproval (Wentzel, et al., 2007). The findings from the current study provide preliminary evidence to suggest that internal motivations such as high afternoon cortisol may influence peer acceptance, teacher closeness, and prosocial behaviours during middle childhood. However, further research is needed to delineate the processes by which daily patterns in salivary cortisol influence children's motivations to engage in supportive peer and teacher relationships and prosocial behaviour.

### **Strengths of the Research**

The current study had a number of strengths. Perhaps most notable, this study was made possible only by the unique participation and joint effort of the school board, principal, students, teachers, and researchers. Presentations to inform children about participating in the study were based on curriculum-based learning outcomes to integrate the research study hypothesis with educational goals. While there has been a call from researchers for this type of classroom-based research (Hamre et al., 2009; Hamre, & Pianta, in press), limited published investigations exist at this time. Most of the existing research examining daily patterns of cortisol and behaviour in school-age children have been limited to summer camps, lab-based settings, or day care contexts with younger children. A second strength of this body of work was the purposeful design of the study to address a number of methodological constraints inherent to investigations examining children's daily cortisol and behaviour including; (1) the examination of various indicators of daily patterns of salivary cortisol in typically developing children in a classroom context, (2) the examination of daily cortisol in relation to specify subtypes of aggression, and (3) the inclusion of prosocial behaviours as an outcome of interest. This research project was one of the first to examine the unique mediating influence of peer and teacher supportive relationships as explanatory process variables by which cortisol is associated with social behaviours in a classroom setting. These findings are consistent with emerging research suggesting that children's relationships with non-familial adults such as peers and teachers have a significant

influence on children's neurobiological development and behavioural development. (National Scientific Council on the Developing Child, 2004). Finally, this research was an empirical examination of the use of different approaches to mediation analyses in child behavioural research.

The current study employed three different methodological approaches to mediation analyses to illustrate the strengths and limitations of each approach. This method was based on emerging evidence suggesting that bootstrapping of indirect effects approach is preferable to traditional approaches to mediation such as the causal steps approach and the Sobel test (see Hayes, 2009; Preacher & Hayes, 2008). Two inconsistent findings were observed among the distinct approaches to mediation analysis that illustrate the methodological drawbacks of the causal steps approach and the Sobel test. The first inconsistency was the observation that the mediating influence of teacher closeness on the association between cortisol and teacher rated proactive aggression was observed with the Sobel test and bootstrapping approaches but not with the causal steps approach. The non-significant finding with the causal steps approach was likely a result of the criteria that a significant direct effect between cortisol and behaviour (path *c*) is required to establish mediation – which was not evident in the present study. However, the indirect paths (path *a* and path *b*) between afternoon cortisol, peer acceptance, teacher closeness and behaviour were statistically significant supporting a mediating effect using the Sobel test and bootstrapping approaches. This finding provides empirical support for the suggestion that relying on statistical significance of the causal paths is not recommended when examining models of mediation (Mackinnon et al., 2002). Despite this knowledge, there remain a number of investigators that still endorse the use of the causal paths approach in mediation analyses (e.g., Rose et al., 2004). Yet, at the same time, there appears to be a shift in the developmental behavioural literature towards the use of the Sobel test (e.g., Morrow et al., 2006). However the

Sobel test also has its limitations, particularly in studies with smaller sample size, as discussed below.

The second inconsistent finding was observed for the mediating influence of peer acceptance on both teacher rated proactive aggression and social aggression. In this case, only the bootstrapping of indirect effects was found to be statistically significant, and no mediating effect was observed with the causal steps or Sobel test approaches. Closer inspection of the individual paths among each variable indicates that no significant direct association was observed between cortisol and teacher rated proactive aggression or social aggression (path *c*). The lack of a main direct effect likely explains why no mediating effect was observed with the causal steps approach. It is likely that the current sample size was not large enough to obtain a significant mediating effect using the Sobel test approach given that the significance of the mediating effect (product *ab*) is based on the standard normal distribution that tends to be normal only in large samples. In contrast, the bootstrapping nonparametric re-sampling procedure does not impose the assumptions of normality of the sampling distribution, and so sample size becomes less relevant (see Hayes, 2009). As such, the bootstrapping approach has greater power to test hypotheses of indirect effects (MacKinnon et al., 2002, 2004), which is a possible explanation for why a significant mediating effect was observed with the bootstrapping approach only. The greater power of the bootstrapping approach has important implications for investigations examining mediating effects in expensive neurobiological studies that tend to have relatively smaller samples.

Consistent with the results of the Sobel test, bootstrapping of the simple indirect effect confirmed the mediating influence of both peer acceptance and teacher closeness on both peer- and teacher-rated prosocial behaviours. Additionally, the bootstrapping approach was consistent with the Sobel test for mediating effects on teacher rated reactive and proactive aggression (see

Table 7 and 8). Overall, these findings suggest that these three approaches to mediation analyses can offer incrementally informative results depending on the nature of the sample data.

### **Limitations and Future Directions**

**Longitudinal research examining biological correlates of behaviour.** This study had several unique strengths however a number of limitations must be acknowledged that provide clear directions for future research. First, it must be acknowledged that this was a cross-sectional study that could not adequately assess directionality among variables. As described earlier, the theoretical basis of this research was founded on the perspective that multiple measurements of basal cortisol in a classroom context represent children's typical everyday HPA axis activity, and that daily HPA axis activity is a predictor of children's behaviours (e.g., El Sheik et al., 2008; Murray-Close et al., 2008). However, it is also plausible that involvement in prosocial or aggressive behaviours leads to changes in levels of supportive relationships with peers and teachers that subsequently influence HPA axis functioning. It is also possible that HPA axis activity is not so much a predictor of behaviour more than it is a risk factor for children who are already displaying behavioural problems. For example, evidence from longitudinal research suggests that low HPA axis activity does not cause the appearance of new behavioural problems but may cause persistence of problem behaviours (see Sondejiker et al., 2008). The results of the research by Sondejiker et al. (2008) revealed that low daily AUC and evening cortisol levels did not predict future disruptive behaviours in school-aged Dutch children (aged 10 to 12 years). Their findings did suggest however that lower evening cortisol significantly predicted parent-reported disruptive behaviours 2 years later in boys who already scored high on disruptive problems. This research is similar to longitudinal research by Smider et al. (2002) and Shirtcliff and Essex (2008) where daily patterns in cortisol are shown to differentially predict concurrent and long-term behavioural and mental health problems. For example, higher afternoon cortisol obtained from young boys (aged 4.5 years) in a home setting is shown to predict higher ratings

of externalizing behaviour one year later but not concurrent behaviour (Smider et al., 2002). Research by Shirtcliff and Essex (2008) suggests that low daily cortisol obtained from fifth grade children (morning cortisol sampled at home and daytime cortisol sampled at school) is associated with their concurrent mental health symptoms, whereas higher cortisol predicts increases in mental health symptoms over the following two years (Shirtcliff & Essex, 2008). However, earlier research by Shirtcliff et al. (2005) suggests that low awakening cortisol is associated with both concurrent and long-term externalizing behaviours, but only long-term internalizing behaviours in boys. Taken together, the results from these longitudinal studies suggest that daily patterns in salivary cortisol in middle childhood may not adequately predict concurrent behavioural problems but could be valuable to identify those children with a poor prognosis once disruptive behaviors are present (Sondeijker et al., 2008). Indeed, Sondeijker and colleagues (2008) propose that HPA axis activity does not represent a risk factor for behaviours but in concert with other risk factors, such as familial stress or adverse peer and teacher relationships, low HPA axis may become more important. Longitudinal studies are needed that document the temporal precedence of daily patterns of cortisol to changes in peer and teacher supportive relationships and behavioural adaptation in middle childhood. Research that measures state changes in daily cortisol while measuring children's state behaviours using classroom observational methods may offer greater insight into how the HPA axis and behaviours are interwoven in real-time (e.g., Hamre et al., 2009). Additionally, longitudinal studies that include information on early rearing environments or quality of care early in life will facilitate better understanding of the origins of HPA axis dysregulation in middle childhood (Cicchetti et al., 2010; Cicchetti & Lynch, 1993; Gunnar & Quevedo, 2007). Longitudinal research exploring biological correlates of behavioural adaptation across the academic year may be of particular interest given the evidence that children's reports of school relatedness (teacher supportiveness, school supportiveness, and loneliness) and school-liking decline from Fall to Spring in school-

age children (see Gest, Welsh, & Domitrovich, 2005), while aggressive behaviours tend to increase over the school year (Aber et al., 2003, Murray-Close et al., 2007). These findings are not surprising given the potential stress of peer social hierarchies, academic demands, and familial expectations associated with school. What is not clear however, is how children's HPA axis (as well as other biological stress systems) typically functions across a school year and whether these changes correlate with behavioural and relational indices. Perhaps some children are biologically predisposed to thrive in a supportive classroom or school context yet languish in stressful, non-supportive environments (Boyce & Ellis, 2005). Of the children who are thriving in school, what aspects of their biological and relational context are driving positive adaptation that can be harnessed in prevention and intervention efforts? For example, school interventions that promote teacher support may buffer children displaying HPA axis dysregulation and aggression. Classroom-based interventions that promote supportive relationships among students and teachers represent a practicable avenue for researchers interested in promoting positive bio-behavioural adaptation in middle childhood.

**Variable- and person-centered approaches to behavioural development.** The current study took a variable-centered approach to understanding the neurobiological correlates of specific subtypes of behaviour in classroom contexts. Variable-centered approaches are useful for identifying processes found to a similar degree in all members of a group (Laursen & Hoff, 2006). However, an important research direction is not only whether some individuals exhibit higher or lower levels of basal cortisol but whether changes in cortisol over time are typical or atypical, with atypical patterns potentially reflecting dysregulation of the HPA axis (Van Ryzin et al., 2009). Future research using advanced techniques such as growth curve modelling will facilitate the characterization of average trajectories of change in basal HPA axis activity to identify variables that predict deviation from that trajectory (e.g., Adam, 2006; Shirtcliff & Essex, 2008). Many developmental research questions however require methods that take a

person-centered approach as well as a variable-centered approach. Person-centered approaches are more appropriate for questions concerning differences among children (see Laursen & Hoff, 2006 for a discussion of both approaches to longitudinal data). A person-centered approach would facilitate a better understanding of the individual differences in children's neurobiological functioning that predict behavioural adaptation. Indeed, an important direction for behavioural researchers appears to be the study of individual differences in neurobiological functioning that predispose children to the benefits and adversities of contextual influences (see Belsky & Pleuss, 2009a; Boyce & Ellis, 2005). For example, emerging research suggests that children exhibiting high levels of acute physiological stress to a laboratory stressor (e.g., skin conductance and heart rate) are more responsive to high or low supportive environments (Boyce & Ellis, 2005; Obradovic, Bush, Stamperdahl, Adler, & Boyce, 2010). Thus, prospective studies that extend over longer periods of time would permit assessment of whether HPA axis dysregulation is responsive to change and if so which factors (e.g., improved or worse classroom supportive environment) bring about change (see Bevans, Cerbone, & Overstreer, 2008). Group-based trajectory modelling is shown to be a more suitable method than growth curve modelling for identifying atypical patterns of change in cortisol over time in distinct subgroups of children (see Van Ryzin et al., 2009). Person-centered approaches may permit identification of the characteristics of children for whom a classroom-based intervention is successful. The degree to which children's daily HPA axis activity and social behaviours are responsive to classroom-based interventions designed to promote peer and teacher supportive relationships is an important unanswered question for future research.

**Measurement of multiple physiological systems.** This study indexed daily patterns in salivary cortisol as a marker for daily HPA axis activity. However, cortisol is only one indistinct peripheral marker of a plethora of possible physiological markers of bio-behavioural development. It is likely that the integrative coordination of multiple aspects of

neurophysiological systems, (i.e., sympathetic nervous system, neuropeptidergic levels of oxytocin) or central nervous system activity (i.e., cortico-releasing hormone activity) are responsible for the observed behavioural phenotypes (see Gunnar & Quevedo, 2007 for a review on the neurobiology of stress and development). For example, research has shown an interaction between measures of the sympathetic nervous system and the HPA axis in predicting childhood behaviour (e.g., Gordis et al., 2006). Lower levels of cortisol may be a result of lower production of cortisol or its hormonal antecedents (e.g., CRH, ACTH), or down regulation of these receptors due to increased levels of circulating hormones (see Gunnar & Vazquez, 2001; Locke, Davidson, Kalin, & Goldsmith, 2009). Moreover, because of counter-regulation in the HPA axis daily patterns in cortisol as well as cortisol in response to an acute stressor may appear normal even when measures higher up in the axis (e.g., ACTH in response to a CRH challenge) are shown to be dysregulated (Cicchetti et al., 2010; Heim et al., 2008). With regards to prosocial behaviour in particular, recent neurobiological research in adults suggests an important role of the neuropeptide oxytocin in regulating HPA axis activity (Bales & Carter, 2007). Oxytocin is shown to increase feelings of trust, sharing, and cooperative behaviours (Carter, 1998). Future research would benefit from concurrent examination of multiple physiological systems to better understand the significance of these systems in predicting behavioural adaptation (Bauer, Quas & Boyce, 2002; Granger & Kivlighan, 2003; Lisonbee et al., 2008).

**Influence of puberty on the developing HPA axis.** Another potential limitation to the current study was the lack of measurement of pubertal status as a risk factor for aggressive behaviours. Research suggests that early adolescence is a vulnerable period for the expression of aggressive behaviours because of the rapid puberty-related neuroendocrine changes that occur (Graber, Lewinsohn, Seeley, & Brooks-Gunn, 1997; Graber, Seeley, Brooks-Gunn, & Lewinsohn, 2004). These changes involve both increases in basal HPA axis activity and changes in HPA axis reactivity during the adolescent years that may place children at risk for mood or

behavioural disorders (Adam, 2006; Gunner, et al., 2009; Shirtcliff et al., 2005). Earlier or later puberty is considered a transitional stressor because being asynchronous with peers can be emotionally arousing. To illustrate, the timing of puberty has been shown to moderate the association between cortisol reactivity and antisocial behaviour in boys aged 8 to 13 years old (e.g., Susman et al., 2010). However, other research has found no influence of puberty on daily cortisol patterns in middle childhood (see Shirtcliff & Essex, 2003). With respect to the current study, it is possible that some of the older children were entering puberty, which may have influenced their daily patterns in cortisol. However, age was included as a covariate for all analyses suggesting that pubertal development in the current sample may not have had a major impact on the findings. Nonetheless, future longitudinal research exploring activity of the HPA axis across middle childhood into adolescence should incorporate measures of pubertal timing.

**Influence of gender.** A significant limitation to the current study was the lack of examination of gender effects. This decision was based on a number of factors. First, this was a relatively unique exploratory study with the precise aim of establishing the associations between daily patterns in salivary cortisol to specific subtypes of aggressive and prosocial behaviour. The strength of the study lay in the robust methodological design including the measurement of salivary cortisol three times a day over four days. However, this also led to a relatively small sample size that did not facilitate modelling of mediating effects by gender. Rather, each analytical model was run controlling for gender, such that the results are generalizable to both boys and girls. However, it is possible that the outcomes are similar for boys and girls but the exact processes by which cortisol and behaviour are linked may be gender specific. Middle childhood is a critical transition period to examine gender effects in daily cortisol patterns and behaviour as this developmental period is characterized by the emergence or consolidation of specific subtypes of aggressive behaviours that may be gender specific such as social aggression (see Neal, 2010; Tyrka et al., 2010). Most existing studies examining gender effects in HPA axis

and behaviour have been conducted in pre-school or adolescent samples (e.g., Booth, Granger & Shirtcliff, 2008). Of these studies some have shown sex differences in measures of salivary cortisol with boys showing lower afternoon cortisol than girls (Klimes-Dougan et al., 2001; Shirtcliff et al., 2005). Others find no difference between boys and girls during middle childhood (e.g., Booth, Granger, & Shirtcliff, 2008; Gunnar et al., 2003). Murray-Close and colleagues (2008) found that HPA axis dysregulation (e.g., steeper or flatter slope) was associated with involvement in physical and relational aggression for children of both genders. The association between gender and behaviour is also variable with some research suggesting a trend for boys to be more physically aggressive (Card et al., 2008), which may be largely accounted for by a small group of boys who are highly aggressive (Campbell, Spieker, Burchinal, & Poe, 2006; Côté et al., 2006; Moffitt, Caspi, Rutter, & Silva, 2001; Tremblay et al., 2004; Underwood et al., 2009). In addition, younger boys are generally socialized towards more aggressive rough and tumble play, as well as to defend against highly aggressive peers (Côté et al., 2006; Hay, 2007). Research suggests that girls develop qualitatively distinct strategies to manipulate their social world resulting in higher rates of social aggression (Card et al., 2008; Crick & Grotpeter, 1995; Crick et al., 1997; Lagerspetz et al., 1988; Vaillancourt, 2005). However, longitudinal evidence suggests that children may follow different developmental trajectories of aggression irrespective of their gender suggesting that individual differences are key in predicting children's adaptation (Fontaine, Carbonneau, Vitaro, Barker, & Tremblay, 2009; Underwood et al., 2009). The findings from the current study represent a starting point for future research interested in examining how the association between daily cortisol patterns and behaviours differ for boys and girls. As discussed earlier, what appears to be an important avenue for future research is to examine how individual differences in children's neurobiological functioning may influence their behavioural development.

### **Measurement of various indicators of daily patterns of cortisol in different settings.**

Another potential avenue to pursue in future research is whether afternoon cortisol would be significantly associated with school-age children's behaviours and social relationships if daily salivary cortisol was sampled in a home setting. The evidence comparing daily cortisol profiles in young children attending day care and adults in the workplace compared to home suggests that school-age children's classroom-based cortisol patterns may differ if sampled on a weekend day in a home setting. For example, Kunz-Ebrecht et al. (2004a) found that the cortisol awakening response (defined as the difference between cortisol levels upon waking and 30 min later) was greater on workdays than weekends in a sample of adults. These results were independent of time since awakening. The authors suggest that cortisol output over the early part of the day may be particularly sensitive to the influence of chronic stress and its anticipation. In fact, evidence suggests that chronic stress is associated with higher morning cortisol in teachers sampled in their workplace environment (Steptoe et al., 2000). Missing from the literature is an exploration of the association between chronic school stress and HPA axis activity in school-age children. The results from the current study suggest that there is a difference in HPA axis functioning between children who have close, supportive relationships at school compared to those children who do not. Chronic school stress as a result of bullying and low quality relationships may predispose children to long-term physical as well as mental health problems (Vaillancourt, et al., 2008).

Another related limitation to the current study was the lack of sampling of the cortisol awakening response (CAR, i.e., increase in cortisol concentration within 20-45 minutes after waking). Previous research has obtained cortisol from children upon awakening as well as during their typical school day with different results associated with early morning or afternoon cortisol (see Shirtcliff & Essex, 2008). Indeed, a review of the CAR and psychosocial factors in adults suggests that the CAR is positively associated with general life stress and negatively associated

with post-traumatic stress syndrome (Chida & Steptoe, 2010). The increasing evidence that specific aspects of the cortisol diurnal rhythm (e.g., CAR, diurnal slope, afternoon cortisol) may differentially index specific behavioural and psychosocial outcomes points to an interesting avenue to pursue in the neurobiological correlates of children's behavioural development.

**Influence of classroom and school-climate.** The findings of this study are limited to individual analyses of children sampled from four classrooms in one school in an urban working-class community. What may be of interest for future research is the influence of classroom effects and school climate on children's neurobiological and behavioural development. For example, a larger research study with more classrooms would facilitate the use of hierarchical linear modelling to examine specific properties of classroom environments such as different instruction techniques or discipline environments that may influence the association between neurobiological functioning and behaviour (e.g., Hamre & Pianta, in press; Pianta et al., 2008; Somersalo, Solantaus, & Almqvist, 2002; Sprott, 2004). Unfortunately, a large number of schools are required to identify ways in which the broader school climate might influence the interrelations among HPA axis, behaviour, and supportive relationships (e.g., 15 to 20 to allow for multi-level models; Gest et al., 2005). Nonetheless, previous research has shown that a school-wide intervention designed to help schools become "a caring community of learners" has positive effects on children's perceptions of school as well as their behaviours. For example, Battistich, Schaps and Wilson (2004) examined the effects of an elementary school-wide intervention in 12 schools on students' connectedness to school and social adjustment during middle school. The findings revealed increased connectedness (e.g., sense of school community, school liking, trust and respect in teachers) and greater prosocial behaviour in program students. What is missing from this research is an examination of how classroom and school climates influence children's neurobiological functioning and behavioural adaptation, and whether some

children are 'biologically susceptible' to the positive effects of a school-based intervention (e.g., Boyce & Ellis, 2005; Obradovic et al., 2010).

**Emotional processes and relational variables.** Another limitation to the current study that offers clear direction for future research is the lack of examination of key variables known to influence children's behaviour such as emotion regulation and emotion processes. Emotional processes are integral to the development of behaviour problems (e.g., Cole & Deater-Deckard, 2009; Spinrad et al., 2006). Children's inability to regulate their emotions, in particular anger, has been associated with increased HPA axis reactivity and reactive but not proactive aggression (Hubbard et al., 2002). Future research that incorporates measures of children's emotion regulation and multiple measures of physiological systems may offer greater insights into the processes by which cortisol and behaviour are linked. For example, real-time observational reports of behaviours and emotions in classroom contexts on the same day as cortisol sampling would permit modelling of emotional and biological correlates of observed incidences of reactive sub-types of aggression, for example, that occur in the 'heat of the moment.' In fact, the current study was limited by the measurement of salivary cortisol within two weeks of the behavioural variables. Although evidence suggests that salivary cortisol is stable across a two-week period, sampling of cortisol at the same time as observational reports of emotions and behaviour will likely offer more information on the biological and emotional processes that underlie specific subtypes of behaviour. In addition to real-time state behavioural observational measures, peer and teacher reports would offer multiple perspectives on behavioural development. This study was based on peer- and teacher-reports of children's behaviour, which may not necessarily map to prosocial or aggressive behaviours. Future studies may want to consider other sources of information such as parental reports to increase the validity of the behavioural constructs examined (see Hamre et al., 2009). This avenue seems especially fruitful given the difference in findings in the current study for peer compared to teacher ratings of

aggressive behaviour. Future research that incorporates measures of social dominance and peer hierarchies may offer interesting insight into the association among HPA axis activity, peer groups, and behaviour (Neal, 2010; Sapolsky, 2005; West et al., 2010).

### **Implications of the Research for Practice**

Our efforts to establish a partnership between educators and researchers enabled this study to address questions regarding the neurobiological correlates of behaviour in an everyday, classroom context that would not have been feasible otherwise. This classroom-based research is in line with emerging neurobiological perspectives on integrating biological processes in prevention and intervention research (Beauchaine et al., 2008). Indeed, evidence from attachment-based interventions in younger children indicates that classroom-based relational interventions may be a fruitful avenue to pursue in school-age children. For example, emerging research has shown promising results in support of environmental interventions, such as high-quality foster care placement, to improve daily HPA axis dysregulation and behaviour in young children with a history of maltreatment (Fisher, Stoolmiller, Gunnar, & Burraston, 2007). Based on evidence that the developing HPA axis is under social regulation during early and middle childhood (Gunnar & Quevedo, 2007), the findings from the current study have practical implications for the promotion of peer and teacher supportive relationships in classrooms during developmentally salient periods such as middle childhood. Programs designed to promote student-teacher supportive relationships are modelled on a strengths-based approach to aggressive behaviour that are shown to improve children's classroom behaviour (e.g., Driscoll & Pianta, 2010; Helker & Ray, 2009; Morrison & Helker, 2010; Sutherland, Conroy, Abrams, & Vo, 2010). Similarly, classroom-based interventions designed to promote peer acceptance have been shown to improve children's behaviour (Witvliet, van Lier, Cuijpers, & Koot, 2009). For example, a recent randomized control trial examined the effectiveness of a classroom-based intervention designed to promote positive peer relations in young children followed from

kindergarten to second grade. Children receiving the intervention demonstrated greater positive peer relationships and improvements in teacher-rated externalizing behaviours, such as disobedience and physical aggression. The results of the research by Witvliet et al. (2009) suggested that the decrease in externalizing behaviour for boys was mediated by greater acceptance by peers. However, a ceiling effect was observed where girls did not display externalizing behaviour prior to the intervention (Witvliet et al., 2009). Future longitudinal studies may want to specify subtypes of aggression that are differentially related to adaptation (see Vitaro et al., 2006), such as social aggression, given the increasing propensity for girls to demonstrate social aggression in middle childhood (Neal, 2010).

Overall, the findings from the current study build on previous research to underscore the importance of enhancing positive peer and teacher relationships in children's classrooms and support the use of positive relational indices, such as peer acceptance and teacher closeness in studies examining promotive factors for children's positive behavioural development. An important direction for future research is the incorporation of neurobiological measures of behavioural development into classroom-based research (Beauchaine, et al., 2008). Research aimed at understanding the dynamic interplay among biological and relational protective factors in classroom contexts may help tailor specific interventions to critical periods such as middle childhood or earlier, during which plasticity is heightened and the intervention may be more efficacious (Cicchetti & Gunnar, 2008; Cicchetti & Lynch, 1993).

### **Concluding Remarks**

The findings from this research contribute to the growing body of knowledge on the associations among children's daily cortisol patterns, social behaviours, and peer and teacher supportive relationships in a classroom context. The results replicate and extend previous empirical research linking cortisol to proactive, reactive, and socially aggressive subtypes of behaviours and prosocial behaviours in a non-clinical sample of children. The unique mediating

influence of peer acceptance and teacher closeness provides important direction for the incorporation of neurobiological and relational-contextual measures of behavioural development in classroom-based research. These preliminary findings are limited to a cross-sectional sample of children in a Western Canadian elementary school. Future longitudinal studies are recommended to identify temporal associations among daily patterns in salivary cortisol, peer and teacher supportive relationships, and behavioural adaptation across middle childhood.

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## **APPENDIX A**

### **Research Ethics Approval**

A-1: The University of British Columbia Behavioural Research Ethics Board (BREB) approval

A-2: Vancouver School Board ethics approval

**A-1: The University of British Columbia Behavioural Research Ethics Board (BREB) approval.**

The University of British Columbia  
Office of Research Services  
**Behavioural Research Ethics Board**  
Suite 102, 6190 Agronomy Road, Vancouver, B.C. V6T 1Z3

## CERTIFICATE OF APPROVAL - MINIMAL RISK

<b>PRINCIPAL INVESTIGATOR:</b> Tim F. Oberlander	<b>INSTITUTION / DEPARTMENT:</b> UBC/Medicine, Faculty of Paediatrics	<b>UBC BREB NUMBER:</b> H06-03862
<b>INSTITUTION(S) WHERE RESEARCH WILL BE CARRIED OUT:</b>		
Institution	Site	
N/A	N/A	
Other locations where the research will be conducted: Hastings Elementary School		
<b>CO-INVESTIGATOR(S):</b> Clyde Hertzman Kimberly A. Schonert-Reichl Joanne Weinberg		
<b>SPONSORING AGENCIES:</b> BC Ministry of Children and Family Development		
<b>PROJECT TITLE:</b> What can your saliva tell you about the way you feel and behave?		
<b>CERTIFICATE EXPIRY DATE: March 2, 2008</b>		
<b>DOCUMENTS INCLUDED IN THIS APPROVAL:</b>	<b>DATE APPROVED:</b> March 2, 2007	
<b>Document Name</b>	<b>Version</b>	<b>Date</b>
<b>Protocol:</b>		
Research Protocol	2	February 21, 2007
<b>Consent Forms:</b>		
School Principal Consent	2	February 21, 2007
Parent/Guardian Consent	3	February 27, 2007
Teacher Consent	2	February 21, 2007
<b>Assent Forms:</b>		
Child Assent	2	February 21, 2007
<b>Questionnaire, Questionnaire Cover Letter, Tests:</b>		
Activities Diary	2	February 22, 2007
My Feelings and Emotions (PANAS-C)	2	February 22, 2007
Who Am I?	2	February 22, 2007
Emotion Scale	2	February 22, 2007
Child Social Behaviour Scale	2	February 22, 2007
Counselor Request	N/A	December 14, 2006
Peer Assessment of Social Behaviour	2	February 22, 2007
My Feelings	2	February 22, 2007
More About Me	2	February 22, 2007

My Life	2	February 22, 2007
Students in My Classroom	2	February 22, 2007
Demographic Questionnaire	2	February 22, 2007
Student-Teacher Relationship Scale	2	February 22, 2007
<b><u>Letter of Initial Contact:</u></b>		
Information Letter to Principal and Teachers	2	February 21, 2007
<b><u>Other Documents:</u></b>		
Teaching Module	N/A	December 14, 2006
Teaching Module Power Point Presentation	N/A	December 14, 2006

The application for ethical review and the document(s) listed above have been reviewed and the procedures were found to be acceptable on ethical grounds for research involving human subjects.

*Approval is issued on behalf of the Behavioural Research Ethics Board  
and signed electronically by one of the following:*

---

Dr. Peter Suedfeld, Chair  
Dr. Jim Rupert, Associate Chair  
Dr. Arminee Kazanjian, Associate Chair  
Dr. M. Judith Lynam, Associate Chair

## A-2: Vancouver School Board Ethics approval.



January 25, 2007

Dr. Tim Oberlander  
Associate Professor  
Department of Paediatrics, UBC  
Centre for Community Child Health Research  
L408 – 4480 Oak St.  
Vancouver, B.C. V6H 3V4

Dear Dr. Oberlander,

Thank you for your research proposal on "What Can Your Saliva Tell You About The Way You Feel and Behave?". On behalf of the VSB Research Committee, please accept this letter as approval for you to complete your research in Vancouver schools. You have permission to contact teachers, parents and students in Vancouver schools. We request that you make your initial contact with the principal of the school to inform them of your study. Please note that teachers and administrators are very busy with many obligations and that schools have the right of refusal to participate in any research studies. Also, the Vancouver School District does not find subjects for researchers.

The VSB Research Committee would be very interested in learning of your results and its implications for students. When your research is completed please send us an abstract of the results.

Thank you for focusing your work within the Vancouver School District. I wish you the best of luck as you proceed with your inquiry.

Sincerely,

Dr. Valerie Overgaard, Associate Superintendent  
Learning Services

## **APPENDIX B**

### **Questionnaire Package**

- B-1 Background information
- B-2 Teacher report of social behaviours
- B-3 Peer nominations of social behaviour
- B-4 Student-teacher closeness
- B-5 Salivary cortisol activity diary

### B-1: Background Information

1. Are you a boy or a girl? (CIRCLE ONE)    BOY    GIRL
2. What grade are you in? (CIRCLE ONE)        4        5        6
3. What is your birthdate? \_\_\_\_\_  
(Month)                      (Day)                      (Year you were born)
4. Which of these adults do you live with MOST OF THE TIME? (Check all the adults you live with).  
Mother                              Grandmother                      1/2 Mom, 1/2 Dad  
Father                                Grandfather                      Foster Parent(s)  
Stepfather                          Stepmother  
Other adults (EXPLAIN, for example, aunt, uncle, mom's boyfriend)  
\_\_\_\_\_
5. Do you live in (Check where you live)?  
House    Apartment    Basement Suite    Townhouse    Condo    Duplex  
Other (Describe)
6. How long have you lived there? \_\_\_\_\_
7. Do you have any brother(s) in your family? (Include stepbrothers)  
No                              Yes    If yes, how old are they?
8. Do you have any sister(s) in your family? (Include stepsisters)  
No                              Yes    If yes, how old are they? \_\_\_\_\_
9. What is the first language you learned at home?  
English    French    Chinese    Punjabi    Vietnamese    Spanish    Tagalog    Other  
\_\_\_\_\_
10. Which language(s) do you speak at home?  
English    French    Chinese    Punjabi    Vietnamese    Spanish    Tagalog  
Other \_\_\_\_\_
11. Which language(s) do you prefer to speak?  
English    French    Chinese    Punjabi    Vietnamese    Spanish    Tagalog  
Other \_\_\_\_\_
12. Does your mum (or female caregiver) work outside the home?  
Yes        No        This does not apply to me  
  
If yes, is it:    part-time        full-time?

13. Does your dad (or male caregiver) work outside the home?

Yes    No    This does not apply to me

If yes, is it:    part-time    full-time?

14. Have you been diagnosed with any of the following? (CHECK BOX IF YOU HAVE)

Diabetes

Asthma

Attention-deficit hyperactivity disorder

Depression

Anxiety

Other (Name) \_\_\_\_\_

15. Do you take medication regularly? (CIRCLE ONE)

YES

NO

16. Please check off any medication that you have taken in the last 2 weeks:

Steroids

Ritalin

Antidepressants

Asthma Inhaler/Puffer (Flovent, Ventolin, etc.)

Other (Name) \_\_\_\_\_

17. How many days a week do you take your medication? (CHECK BOX)

1 day

2 days

3 days

4 days

5 days

6 days

7 days

18. How many times do you take your medication in one day? (CHECK BOX)

1 time a day

2 times a day

3 times a day

more than 3 times a day

## B-2: Teacher Report of Social Behaviours

Please consider the descriptions contained in each of the following items below and rate the extent to which each of these descriptions applies to this child, particularly in the context of his/her behaviour with peers. Using the answers “never or not true,” “sometimes or somewhat true,” and “often or very true,” how often would you say that this child . . . (Mark the circle corresponding to your answer, mark only one response per item.)

	<b>Never or Not true</b>	<b>Sometimes or Somewhat true</b>	<b>Often or Very true</b>
1. Shows sympathy to someone who has made a mistake.	○	○	○
2. Will try to help someone who has been hurt.	○	○	○
3. Gets into many fights.	○	○	○
4. Threatens or bullies other children to get his/her own way.	○	○	○
5. Volunteers to help someone clear up a mess that someone else has made.	○	○	○
6. When mad at someone, tries to get others to dislike that person.	○	○	○
7. Destroys things belonging to his/her family, or other children.	○	○	○
8. When teased or threatened, he/ she gets angry easily and strikes back.	○	○	○
9. If there is a quarrel or a dispute, will try to stop it.	○	○	○
10. When mad at someone, becomes friends with another as revenge.	○	○	○
11. Offers to help other children (friend, brother or sister) who are having difficulty with a task.	○	○	○
12. Claims that other children are to blame in fight and feels like they started the trouble.	○	○	○
13. When another child accidentally hurts him/her (such as by bumping into him/her), assumes that the other child meant to do it, and reacts with anger and fighting.	○	○	○
14. When mad at someone, says bad things behind the other's back.	○	○	○
15. Comforts a child (friend, brother or sister) who is crying or upset.	○	○	○
16. Plays mean tricks.	○	○	○
17. Threatens people.	○	○	○
18. Spontaneously helps to pick up objects which another child has dropped (e.g., pencil, book).	○	○	○

	<b>Never or Not true</b>	<b>Sometimes or Somewhat true</b>	<b>Often or Very true</b>
19. Is cruel, bullies, or is mean to others.	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
20. Uses physical force, or threatens to use force, to dominate other children.	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
21. When mad at someone, says to others, "Let's not be with him/her."	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
22. Kicks, bites, hits other children.	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
23. Plans aggressive acts.	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
24. Helps other children (friend, brother or sister) who are feeling sick.	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
25. Will invite bystanders to join in a game.	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
26. Careful to protect self when aggressive.	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
27. Gets other children to gang up on a peer that he/she does not like.	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
28. When mad at someone, tells the other one's secrets to a third person.	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
29. Picks on smaller kids.	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
30. Has hurt others to win a game.	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
31. Hides aggressive acts.	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
32. Takes the opportunity to praise the work of less able children.	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
33. Can control own behaviour when aggressive.	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

### B-3: Peer Nominations of Social Behaviour

**DIRECTIONS:**

On the following pages, is a list of your classmates. We would like to get some information about your feelings about them and their behaviours. Please follow the directions carefully.

YOU MAY CIRCLE YOUR OWN NAME if you believe the description applies to you.

In each of these long boxes, circle the names of: (Do one box at a time.)

<b>1) Students who <u>share and cooperate.</u></b>	<b>2) Students who <u>start fights.</u></b>	<b>3) Students who <u>help other kids when they have a problem.</u></b>	<b>4) Students who <u>break the rules and do things they're not supposed to do.</u></b>
Student A	Student A	Student A	Student A
Student B	Student B	Student B	Student B
Student C	Student C	Student C	Student C
Student ...Z	Student ...Z	Student ...Z	Student ...Z

<b>5) Students who <u>understand other kids' points of view.</u></b>	<b>6) Students who <u>talk behind other people's backs.</u></b>	<b>7) Students who <u>when mad at someone say, "Let's not be his/her friend."</u></b>	<b>8) Students who <u>are bullies.</u></b>
Student A	Student A	Student A	Student A
Student B	Student B	Student B	Student B
Student C	Student C	Student C	Student C
Student ...Z	Student ...Z	Student ...Z	Student ...Z

**B-3: Peer Nominations of Social Behaviour**

<b>9) Students who <u>get angry easily and fight back when teased.</u></b>	<b>10) Students who <u>you would like to be in school activities with.</u></b>	<b>11) Students who <u>play mean tricks or make plans to hurt others.</u></b>	<b>12) Students who <u>get mad at kids who hurt them by accident.</u></b>
Student A	Student A	Student A	Student A
Student B	Student B	Student B	Student B
Student C	Student C	Student C	Student C
Student ...Z	Student ...Z	Student ...Z	Student ...Z

<b>13) Students who <u>get other students to gang up on a classmate.</u></b>
Student A
Student B
Student C
Student ...Z

<b>14) Students who <u>you can trust.</u></b>
Student A
Student B
Student C
Student ...Z

<b>15) Students who <u>include other kids in their group when they are playing.</u></b>
Student A
Student B
Student C
Student ...Z

### B-4: Student-Teacher Closeness

Please reflect on the degree to which each of the following statements currently applies to your relationship with this child. Using the point scale below, CIRCLE the appropriate number for each item.

	Definitely does not apply	Does not really apply	Neutral, not sure	Applies somewhat	Definitely applies
I share an affectionate, warm relationship with this child.	1	2	3	4	5
If upset, this child will seek comfort from me.	1	2	3	4	5
This child is uncomfortable with physical affection or touch from me.	1	2	3	4	5
This child values his/her relationship with me.	1	2	3	4	5
When I praise this child, he/she beams with pride.	1	2	3	4	5
This child spontaneously shares information about himself/herself.	1	2	3	4	5
This child tries to please me.	1	2	3	4	5
It is easy to be in tune with what this child is feeling.	1	2	3	4	5
I've noticed this child copying my behavior or ways of doing things.	1	2	3	4	5
This child openly shares his/her feelings and experiences with me.	1	2	3	4	5
My interactions with this child make me feel effective and confident.	1	2	3	4	5

### B-5: Salivary Cortisol Activity Diary

#### Student Diary 9:15 AM:

1. What time did you **wake up** this morning? 6:00 6:30 7:00 7:30 8:00 8:30  
9:00am
2. Did you **brush your teeth**? YES NO DON'T REMEMBER
3. Did you have **breakfast** this morning? YES NO  
If yes, at what time? 6:00 6:30 7:00 7:30 8:00 8:30 9:00am  
What did you **eat**? cereal toast eggs bacon pancakes muffin yogurt fruit other:  
\_\_\_\_\_
4. Have you had anything to **drink** this morning? YES NO  
If yes, at what time? 6:00 6:30 7:00 7:30 8:00 8:30 9:00am  
What did you have to drink? milk juice water pop chocolate-milk other:\_\_\_\_\_
5. Have you taken any **pills or medicine** this morning? YES NO  
If yes, at what time? 6:00 6:30 7:00 7:30 8:00 8:30 9:00am  
What did you take?\_\_\_\_\_

#### Student Diary 11:45 AM:

1. Did you have something to **eat** at recess or since then? YES NO  
If yes, at what time? 9:30 10:00 10:30 11:00 11:30am  
What did you have to eat? fruit yogurt granola bar chips muffin cookie other:  
\_\_\_\_\_
2. Did you have something to **drink** at recess or since then? YES NO  
If yes, at what time? 9:30 10:00 10:30 11:00 11:30am  
What did you have to drink? milk juice water pop chocolate-milk  
other:\_\_\_\_\_

## B-5: Salivary Cortisol Activity Diary

### Student Diary 2:45 PM:

1. Did you have something to **eat** at lunch? YES NO  
If yes, at what time? 12:00 12:30 1:00 1:30 2:00 2:30pm  
What did you have to eat? sandwich soup pizza hotdog pasta/rice  
other:\_\_\_\_\_
2. Did you have something to **drink** at lunch? YES NO  
If yes, at what time? 12:00 12:30 1:00 1:30 2:00 2:30pm  
What did you have to drink? milk juice water pop chocolate-milk other:
3. Have you had a **snack** since then? YES NO  
If yes, at what time? 12:00 12:30 1:00 1:30 2:00 2:30pm  
What did you have? fruit yogurt granola bar chips muffin cookie  
other:\_\_\_\_\_
4. Have you taken any **pills or medicine** while at school today? YES NO  
If yes, at what time? 9:30 10:00 10:30 11:00 11:30 12:00 12:30 1:00  
1:30 2:00 2:30  
What did you take?\_\_\_\_\_

## **APPENDIX C**

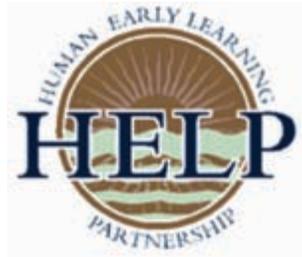
### **Consent and Assent Forms**

C-1 Parental consent

C-2 Child assent

C-3 Teacher consent

*University of British Columbia  
Department of Pediatrics  
British Columbia Children's and  
Women's Health Center*



February 21, 2007.

Dear Parent/Guardian,

We are writing to request permission for your child to participate in a research project that we are conducting entitled **What can your saliva tell you about the way you feel and behave?** Our research program is a combined effort within the Human Early Learning Partnership (HELP) involving the University of British Columbia's Departments of Pediatrics, Education, Anatomy, Medicine and Psychology and has the approval of the Vancouver School Board.

**Purpose:** This project will enable us to see how children respond to everyday challenges (as measured by cortisol, alpha amylase and S100 beta protein in saliva) and what this tells us about health and behaviour in school-age children.

**Procedure:** If you and your son/daughter agree to participate, we will work closely with classroom teachers to schedule study sessions during the child's regular school day with his/her teacher. We will ensure that all data collection sessions are carefully coordinated with the classroom teacher and will in no way interfere with classroom activities or essential learning. Those students not participating in the study will be doing regular school work consistent with their classroom schedule.

On the days your son/daughter participates in the study the first session will be for your child to answer a demographic questionnaire and some questionnaires about behaviour and their feelings and emotions. One of these questionnaires will ask your child to answer questions about the behaviour of the students in their classroom. Two weeks later we will return to your child's classroom over 4 consecutive days to obtain three saliva samples each day: when he/she first comes to school (9 am), before lunch (12 noon), and at school dismissal (3 pm). These samples will be obtained by asking your son/daughter to place an oral swab under his/her tongue for approximately 1 minute and then, with a gloved hand, place the swab in a tube. Before each saliva collection your son/daughter will be asked to complete an activities diary describing what he/she has had to eat or drink during the day, whether he/she is taking any medication and answer a brief questionnaire about his/her feelings.

Your son/daughter's classroom teacher will also be asked to fill out two questionnaires on each student who is participating in the study. One questionnaire is a measure of social behaviour and the other is a rating of student-teacher relationship.

## C-1: Parental Consent

**Risks:** There are no known risks or side effects of the questionnaires or collecting saliva.

**Confidentiality:** Any information resulting from this research study will be kept strictly confidential. All documents will be identified only by a code number and kept in a secured information system and locked filing cabinet. The identity of the participants in this study (both teachers and your son/daughter) will be entirely confidential. No information that discloses your child's identity will be released or published without specific consent to the disclosure. Your child's identity will not be identified by name in any reports of the completed study.

Copies of the relevant data, which identify the participants only by code number, may be published in scientific journals, but no participant will be identified by name. However, research records identifying participants may be inspected in the presence of the Investigator or his or her designate by representatives of the UBC Research Ethics Board for the purpose of monitoring the research.

**Contacts:** The chief investigator would be happy to answer any questions at any time regarding this study to ensure that the participants understand completely what it involves. Should you have any questions, please do not hesitate to call the Research Assistant at (604) 875-2000 (6921). If you have any concerns about your participation in this research programme please call the Research Subject's Information Line at the UBC Office of Research Services at (604) 822-8598.

We would appreciate it if you could indicate on the slip provided on the attached page whether or not your son/daughter has your permission to participate. Would you please sign and date the attached slip where indicated and have your son/daughter return the bottom portion of the slip to school tomorrow.

Thank you very much for considering this request.

Sincerely,

Dr. Tim Oberlander  
Associate Professor  
Department of Pediatrics  
University of British Columbia

Dr. Kimberly Schonert-Reichl  
Associate Professor  
Faculty of Education  
University of British Columbia

### **Co-Investigators**

Dr. Clyde Hertzman, *Professor, Faculty of Medicine, UBC*  
Dr. Joanne Weinberg, *Professor, Department of Anatomy, UBC*  
HELP Psychobiology Research Group

## C-1: Parental Consent

### PARENT CONSENT FORM

**Study Title:** What can your saliva tell you about the way you feel and behave?  
**Principal Investigator:** Dr. Tim Oblerlander, *Associate Professor, Department of Paediatrics, University of British Columbia, Vancouver, B.C.*  
**Funding Agency:** Human Early Learning Partnership (HELP)

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#### ***KEEP THE ABOVE LETTER AND THIS PORTION FOR YOUR RECORDS***

I understand that my child's participation in the above study is entirely voluntary, and that I or my child may refuse to participate, or I or my child is free to withdraw from the study at any time. Refusing to participate or withdrawing from the study will in no way interfere with the education or services provided to my child or me by the Vancouver School Board, nor will it result in any consequences to my child's involvement with Children's and Women's Hospital. I have received a copy of this consent form for my own records. I consent to my child's participation in this study and in signing this document I am, in no way, waiving the legal rights of myself or my child.

I have read and understand the attached letter regarding the study entitled "What can your saliva tell you about the way you feel and behave?". I have also kept copies of both the letter describing the study and this permission slip.

\_\_\_\_\_ Yes, I agree to my son/daughter participating in this project **What can your saliva tell you about the way you feel and behave?**

\_\_\_\_\_ No, my son/daughter does not have my permission to participate.

---

*Parent's Signature*

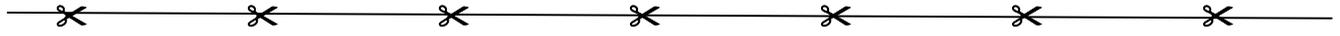
*Printed Name*

*Date*

---

*Son or Daughter's Name*

**C-1: Parental Consent**



***DETACH HERE AND RETURN TO SCHOOL***

I understand that my child’s participation in the above study is entirely voluntary, and that I or my child may refuse to participate, or may withdraw from the study at any time without any consequences to my child’s involvement with Children’s and Women’s Hospital. I have received a copy of this consent form for my own records. I consent to my child’s participation in this study and in signing this document I am, in no way, waiving the legal rights of myself or my child.

I have read and understand the attached letter regarding the study entitled “**What can your saliva tell you about the way you feel and behave**”. I have also kept copies of both the letter describing the study and this permission slip.

\_\_\_\_\_ Yes, I agree to my son/daughter participating in this project.

\_\_\_\_\_ No, my son/daughter does not have my permission to participate.

---

*Parent’s Signature*

*Printed Name*

*Date*

---

*Son or Daughter’s Name*

## C-2: Child Assent

University of British Columbia  
Department of Pediatrics  
British Columbia Children's and Women's  
Health Center



### CHILD ASSENT FORM

#### *What can your saliva tell you about the way you feel and behave?*

##### ***Why are we doing this project?***

This project is to learn about how your everyday activities in the classroom affect substances found in your saliva.

##### ***What will happen during this project?***

Throughout the 4 days you participate, we will collect 3 saliva samples: when you first come to school (9 am), before lunch (11:45 am), at school dismissal (2:45 pm). The sample will be taken by placing an oral swab under your tongue for approximately 1 minute and then, with a gloved hand, placing the swab in a tube. You will be shown how to do this before we get you to take the sample.

Before each collection you will be asked to complete an activities diary describing what you have had to eat or drink or any medicine that you take and a questionnaire that describes how you feel at that moment.

Two weeks before the saliva collection, you will spend some time in your classroom with a research assistant filling out questionnaires about how you feel.

You can choose not to answer questions in the questionnaires that you may not feel comfortable answering. If you choose to leave a question blank you can still stay in the study.

##### ***Can anything bad happen to me?***

Helping with this project will not hurt you or make you sick. The oral swabs used to collect saliva will taste like paper.

##### ***Who will know that I am taking part?***

We will not show your name to anyone and nothing on the computer identifies who you are. We will use a secret code on all the information that you give to us. When we write a report of this project, we will not use your name or initials.

**C-2: Child Assent**

***Who can I talk to if I have any questions?***

If you have any questions at any time during this project, you may ask the researcher who will be with you. Your mother or father can also contact us with your questions.

If you have any questions about this project or about the way you are feeling after the project, you should phone Dr. Tim Oberlander or one of his Research Assistants at (604) 875-2000 (6921). If you are worried about how you were treated during the project, you should contact the Research Subject's Information Line at the UBC Office of Research Services at (604) 822-8598.



***My Assent to:***

***What can your saliva tell you about the way you feel and behave?***

I am taking part in this project because I want to.

If I want to stop being in this project, it is okay and no one will get angry. I just need to tell my teacher or the research assistant that I do not want to do it anymore.

I have had enough time to read this form, to ask questions about this project and to talk to my parents/guardians. All my questions have been answered and I have received a copy of this form to keep.

---

*Your Printed Name*

*Your Signature*

*Date*

### C-3: Teacher Consent

*University of British Columbia  
Department of Pediatrics  
British Columbia Children's  
and Women's Health Center*



April 5, 2007.

Dear Teacher,

We are writing you to ask you and your students to participate in the next phase of our project studying relationships between patterns of everyday autonomic arousal, emotional well being and classroom context. This phase of our work is entitled **What can your saliva tell you about the way you feel and behave?**

We plan to examine relationships between everyday patterns of the non-invasive salivary biomarkers: cortisol, alpha-amylase (stress related chemical in saliva), levels of the S100 beta protein and typical childhood feelings and social behaviours. Our research programme is a combined effort within the Human Early Learning Partnership (HELP) involving the University of British Columbia's Departments of Pediatrics, Education, Anatomy, Medicine and Psychology and has the approval of the Vancouver School Board.

To ensure that the school, students and teachers benefit we have also prepared a teaching module about how stress can have an affect on the way we act and feel. This teaching module is consistent with VSB curriculum.

**Background:** Why children behave and develop the way they do is a core question facing clinicians and researchers alike. Sometimes we ask this question because behaviour and development deviate from typical trajectories, other times we ask this because development is on track and we want to know how it got that way, even when circumstances are unexpected. While we are able to characterize childhood behavioural disorders into clinically recognizable patterns, in reality behaviours are only weakly predicative of concurrent or subsequent psychopathology. To further understand childhood behaviours, physiological measures of stress reactivity have been studied as a "window" into the brain that might underlie these early indicators of development during childhood. Studies have shown links between physiological stress responses, temperament and problematic behaviours in childhood and these relationships may tell us something about future health.

Previously we studied relationships between the stress hormone cortisol, social behaviour and autonomic nervous system responses to stress in children in grades 5 to 7. In this phase of our work we are shifting our focus to examine relationships between everyday patterns in cortisol, alpha-amylase, levels of the S100 beta protein, and typical childhood feelings and social behaviours. This multi-phased research programme is led by Dr. Tim Oberlander, Dr. Kim

### C-3: Teacher Consent

Schonert-Reichl and colleagues in the Human Early Learning Partnership, University of British Columbia and at the Centre for Community Child Health Research, Children's and Women's Health Centre and Child and Family Research Institute. Below we describe our project and the educational benefits we expect that this work will bring to the school, teachers and students who participate. We believe that participatory research is an essential component to undertaking studies of this type in the classroom.

**Purpose:** This project will enable us to see how children respond to everyday challenges (as measured by alpha amylase, S100 beta protein and cortisol in saliva) and what this tells us about health and behaviour in school-age children.

**Procedure:** Research assistants will require one lesson period on the day the teaching module is presented and letters of consent are handed out. About a week later the study personnel will return to the classroom for a lesson period to administer and collect questionnaire measures of feelings and emotions from students. The saliva collection period of the study will occur 1-2 weeks after the questionnaire block. During this phase of the study, research assistants will visit the classroom 4 consecutive days throughout the week collecting samples 3 times a day at 9:00 am, 11:45 am and 2:45 pm (each sample collection time will take approximately 15 minutes).

Classroom Teachers will be asked to fill out two teacher assessment questionnaires (Social Behaviour Scale and Student-Teacher Relationship Scale) for each student, review the content of the study lesson to be presented, provide class lists, coordinate appropriate times with the research assistant for when the stages of the study can be carried out, and collect permission forms from students. A TOC will be provided to cover your classroom while you complete the questionnaires (we will arrange this with you in the near future).

Our staff will carry out all aspects of student recruitment, participation, data collection and teaching. At least two Research Assistants will be present during all aspects of data collection. They will provide all equipment and teaching materials. Beyond discussion about the study and recruiting the children, the Research Assistants will not interfere with any classroom procedures. Those students not participating in the study will be doing regular school work consistent with their classroom schedule.

**Risks:** There are no known risks or side effects of the questionnaires or collecting saliva.

**Confidentiality:** Any information resulting from this research study will be kept strictly confidential. All documents will be identified only by code number and kept in a secured information system and locked filing cabinet. The identity of the participants in this study (both teachers and students) will be entirely confidential. No information that discloses the child's identity will be released or published without specific consent to the disclosure. Participant identity will not be identified by name in any reports of the completed study.

### C-3: Teacher Consent

Copies of the relevant data, which identify the participants only by code number, may be published in scientific journals, but no participant will be identified by name. However, research records identifying participants may be inspected in the presence of the Investigator or his or her designate by representatives of the UBC Research Ethics Board for the purpose of monitoring the research.

**Contacts:** The chief investigator would be happy to answer any questions at any time regarding this study to ensure that the participants understand completely what it involves. Should you have any questions, please do not hesitate to call the Research Assistant at (604) 875-2000 (6921). If you have any concerns about your participation in this research programme please call the Research Subject's Information Line at the UBC Office of Research Services at (604) 822-8598.

**Remuneration:** An honorarium of \$150.00 will be given in recognition of your help with our project.

We would appreciate it if you could indicate on the slip provided on the attached page whether or not you give your permission to participate. Would you kindly sign and date the attached slip where indicated. We will arrange a time with you to come pick up the permission slips that the students have returned.

Thank you very much for considering this request.

Sincerely,

Dr. Tim Oberlander  
Associate Professor  
Department of Pediatrics  
University of British Columbia

Dr. Kimberly Schonert-Reichl  
Associate Professor  
Faculty of Education  
University of British Columbia

#### **Co-Investigators**

Dr. Clyde Hertzman, *Professor, Faculty of Medicine, UBC*

Dr. Joanne Weinberg, *Professor, Department of Anatomy, UBC*

HELP Psychobiology Research Group

### C-3: Teacher Consent

**Study Title:** What can your saliva tell you about the way you feel and behave?  
**Principal Investigator:** Dr. Tim Oberlander, Associate Professor, Department of  
Paediatrics, University of British Columbia, Vancouver,  
**Funding Agency:** Human Early Learning Partnership (HELP)

---

#### ***KEEP THIS PORTION FOR YOUR RECORDS***

I understand that my participation in the above study is entirely voluntary, and that I may refuse to participate, or I am free to withdraw from the study at any time without any consequences to my job or professional standing. I have received a copy of this consent form for my own records. I consent to my participation in this study and in signing this document I am, in no way, waiving my legal rights.

I have read and understand the attached letter regarding the study entitled “**What can your saliva tell you about the way you feel and behave?**” I have also kept copies of both the letter describing the study and this permission slip.

\_\_\_\_\_ Yes, I will participate.

\_\_\_\_\_ No, I will not participate.

---

*Teacher's Signature*

*Printed Name*

*Date*

### C-3: Teacher Consent



***DETACH HERE AND STUDY PERSONNEL WILL CONTACT THE SCHOOL FOR PICKUP***

School Name: \_\_\_\_\_

I understand that my participation in the above study is entirely voluntary, and that I may refuse to participate, or I am free to withdraw from the study at any time without any consequences to my job or professional standing. I have received a copy of this consent form for my own records. I consent to my participation in this study and in signing this document I am, in no way, waiving my legal rights.

I have read and understand the attached letter regarding the study entitled “Working Together: Your Brain and Saliva”. I have also kept copies of both the letter describing the study and this permission slip.

\_\_\_\_\_ Yes, I will participate.

\_\_\_\_\_ No, I will not participate.

---

*Teacher's Signature*

*Printed Name*

*Date*

## **APPENDIX D**

### **Unstandardized Regression Coefficient Tables**

Table D1. Multiple regression analysis of afternoon cortisol on the mediator, peer acceptance (path *a*), controlling for sex and age.

	Peer Acceptance							
	Step 1				Step 2			
	<i>B</i>	<i>SE B</i>	$\beta$	95% <i>CI</i>	<i>B</i>	<i>SE B</i>	$\beta$	95% <i>CI</i>
Sex	-.02	.02	-.08	-.07, .03	-.03	.02	-.12	-.08,.02
Age	.06	.02	.33	.02, .10	.05	.02	.25	.01, .09
Afternoon Cortisol					.266	.095	.29	.08,.46
$\Delta R^2$	.11				.08**			
$R^2$	.11				.19**			

Note: *N* = 89; \*\* *p* < .01; *CI* = 95% Confidence Intervals Lower, Upper

Table D2. Multiple regression analyses testing the mediating effect of peer acceptance on the relations between afternoon cortisol and peer-nominated behaviour (path  $c'$ ), controlling for sex and age: Prosocial behaviour

	Peer-nominated Prosocial Behaviour											
	Step 1				Step 2				Step 3			
	<i>B</i>	<i>SE B</i>	$\beta$	<i>CI</i>	<i>B</i>	<i>SE B</i>	$\beta$	<i>CI</i>	<i>B</i>	<i>SE B</i>	$\beta$	<i>CI</i>
Sex	.15	.03	.42	.08, .22	.14	.03	.39	.07, .21	.17	.02	.47	.12, .22
Age	.09	.03	.29	.03, .14	.07	.03	.24	.01, .13	.02	.02	.08	-.02, .07
Afternoon Cortisol					.28	.13	.20	.01, .54	.02	.10	.02	-.18, .22
Peer Acceptance									.96	.11	.64	.74, 1.18
$\Delta R^2$	.27***				.04*				.33***			
$R^2$	.27				.31				.64			
Aroian test	$z = 2.65***$											

Note:  $N = 88$ ; \*  $p < .05$ ; \*\*  $p \leq .01$ ; \*\*\*  $p < .001$ ; CI = 95% Confidence Intervals Lower, Upper

Table D3. Multiple regression analyses testing the mediating effect of peer acceptance on the relations between afternoon cortisol and peer-nominated behaviour (path  $c'$ ), controlling for sex and age: Reactive aggression

	Peer-nominated Reactive Aggression											
	Step 1				Step 2				Step 3			
	<i>B</i>	<i>SE B</i>	$\beta$	<i>CI</i>	<i>B</i>	<i>SE B</i>	$\beta$	<i>CI</i>	<i>B</i>	<i>SE B</i>	$\beta$	<i>CI</i>
Sex	-.10	.04	-.25	-.17, -.02	-.08	.04	-.21	-.15, -.01	-.09	.04	-.23	-.16, -.02
Age	-.12	.03	-.37	-.18, -.06	-.09	.03	-.29	-.15, -.03	-.08	.03	-.24	-.14, -.01
Afternoon Cortisol					-.47	.14	-.32	-.76, -.19	-.38	.15	-.26	-.68, -.09
Peer Acceptance									-.33	.17	-.20	-.66, .003
$\Delta R^2$	.21***				.09***				.03*			
$R^2$	.21				.30				.34			
Aroian test	$z = -1.54$											

Note:  $N = 87$ . \*  $p = .052$ ; \*\*\* $p \leq .001$ ; *CI* = 95% Confidence Intervals Lower, Upper

Table D4. Multiple regression analyses testing the mediating effect of peer acceptance on the relations between afternoon cortisol and peer-nominated behaviour (path  $c'$ ), controlling for sex and age: Proactive aggression

	Peer-nominated Proactive Aggression											
	Step 1				Step 2				Step 3			
	<i>B</i>	<i>SE B</i>	$\beta$	<i>CI</i>	<i>B</i>	<i>SE B</i>	$\beta$	<i>CI</i>	<i>B</i>	<i>SE B</i>	$\beta$	<i>CI</i>
Sex	-.10	.04	-.29	-.17, -.03	-.09	.04	-.24	-.16, -.02	-.09	.04	-.26	-.16, -.02
Age	-.05	.03	-.18	-.11, .01	-.03	.03	-.10	-.09, .03	-.02	.03	-.06	-.08, .04
Afternoon Cortisol					-.44	.14	-.32	-.71, -.16	-.37	.15	-.27	-.66, -.08
Peer Acceptance									-.25	.16	.16	-.56, .07
$\Delta R^2$	.12**				.09**				.02			
$R^2$	.12				.21				.23			
Aroian test	$z = -1.29$											

Note:  $N = 88$ ; \*\* $p < .01$ ; CI = 95% Confidence Intervals Lower, Upper

Table D5. Multiple regression analyses testing the mediating effect of peer acceptance on the relations between afternoon cortisol and peer-nominated behaviour (path  $c'$ ), controlling for sex and age: Social aggression

	Peer-nominated Social Aggression											
	Step 1				Step 2				Step 3			
	<i>B</i>	<i>SE B</i>	$\beta$	<i>CI</i>	<i>B</i>	<i>SE B</i>	$\beta$	<i>CI</i>	<i>B</i>	<i>SE B</i>	$\beta$	<i>CI</i>
Sex	.04	.04	.10	-.03, .11	.05	.04	.15	-.02, .12	.04	.04	.13	-.03, .11
Age	-.04	.03	-.14	-.10, .02	-.01	.03	-.05	-.07, .05	.001	.03	.003	-.06, .06
Afternoon Cortisol					-.43	.14	-.33	-.71, -.15	-.35	.15	-.27	-.64, -.06
Peer Acceptance									-.28	.16	-.20	-.61, .04
$\Delta R^2$	.03				.10**				.03			
$R^2$	.03				.13				.16			
Aroian test	$z = -1.41$											

Note:  $N = 87$ ; \*  $p = .051$ ; \*\*  $p < .01$ ; CI = 95% Confidence Intervals Lower, Upper

Table D6. Multiple regression analyses testing the mediating effect of peer acceptance on the relations between afternoon cortisol and teacher-rated behaviour (path  $c'$ ), controlling for sex and age: Prosocial

	Teacher-rated Prosocial behaviour											
	Step 1				Step 2				Step 3			
	<i>B</i>	<i>SE B</i>	$\beta$	<i>CI</i>	<i>B</i>	<i>SE B</i>	$\beta$	<i>CI</i>	<i>B</i>	<i>SE B</i>	$\beta$	<i>CI</i>
Sex	.33	.11	.31	.12, .54	.26	.10	.25	.07, .46	.31	.09	.29	.13, .49
Age	.24	.09	.27	.06, .41	.16	.08	.15	-.03, .30	.06	.08	.07	-.10, .22
Afternoon Cortisol					1.79	.39	.43	1.02, 2.56	1.39	.38	.34	.64, 2.14
Peer Acceptance									1.49	.42	.33	.67, 2.32
$\Delta R^2$	.17***				.17**				.09***			
$R^2$	.17				.34				.43			
Aroian test	$z = 2.16^*$											

Note:  $N = 89$ ; \*  $p < .05$ ; \*\*\*  $p \leq .001$ ; CI = 95% Confidence Intervals Lower, Upper

Table D7. Multiple regression analyses testing the mediating effect of peer acceptance on the relations between afternoon cortisol and teacher-rated behaviour (path  $c'$ ), controlling for sex and age: Reactive aggression

	Teacher-rated Reactive aggression											
	Step 1				Step 2				Step 3			
	<i>B</i>	<i>SE B</i>	$\beta$	<i>CI</i>	<i>B</i>	<i>SE B</i>	$\beta$	<i>CI</i>	<i>B</i>	<i>SE B</i>	$\beta$	<i>CI</i>
Sex	-.24	.12	-.21	-.48, .01	-.19	.12	-.16	-.43, .05	-.25	.11	-.21	-.47, -.02
Age	-.09	.10	-.10	-.29, .11	-.02	.10	-.02	-.22, .19	.08	.10	.08	-.11, .27
Afternoon Cortisol					-1.34	.48	-.30	-2.30, -.39	-.82	.46	-.18	-1.74, .11
Peer Acceptance									-1.97	.51	-.40	-2.99, -.96
$\Delta R^2$	.05				.08**				.13***			
$R^2$	.05				.13				.27			
Aroian test	$z = -2.22^*$											

Note:  $N = 88$ ; \*  $p < .05$ ; \*\*  $p < .01$ ; \*\*\*  $p \leq .001$ ; CI = 95% Confidence Intervals Lower, Upper

Table D8. Multiple regression analyses testing the mediating effect of peer acceptance on the relations between afternoon cortisol and teacher-rated behaviour (path  $c'$ ), controlling for sex and age: Proactive aggression

	Teacher-rated Proactive Aggression											
	Step 1				Step 2				Step 3			
	<i>B</i>	<i>SE B</i>	$\beta$	<i>CI</i>	<i>B</i>	<i>SE B</i>	$\beta$	<i>CI</i>	<i>B</i>	<i>SE B</i>	$\beta$	<i>CI</i>
Sex	-.01	.01	-.08	-.03, .02	-.01	.01	-.06	-.03, .02	-.01	.01	-.08	-.03, .02
Age	.01	.01	.12	-.01, .03	.01	.01	.15	-.01, .03	.02	.01	.20	-.003, .02
Afternoon Cortisol					-.05	.05	-.12	-.15, .04	-.03	.05	-.07	-.13, .07
Peer Acceptance									-.09	.05	-.19	-.20-.02
$\Delta R^2$	.02				.01				.03			
$R^2$	.02				.03				.06			
Aroian test	$z = -1.33$											

Note:  $N = 88$ ; CI = 95% Confidence Intervals Lower, Upper

Table D9 . Multiple regression analyses testing the mediating effect of peer acceptance on the relations between afternoon cortisol and teacher-rated behaviour (path  $c'$ ), controlling for sex and age: Social aggression

	Teacher-rated Social Aggression											
	Step 1				Step 2				Step 3			
	<i>B</i>	<i>SE B</i>	$\beta$	<i>CI</i>	<i>B</i>	<i>SE B</i>	$\beta$	<i>CI</i>	<i>B</i>	<i>SE B</i>	$\beta$	<i>CI</i>
Sex	.04	.09	.05	-.13, .21	.05	.09	.06	-.12, .22	.02	.09	.03	-.15, .19
Age	.08	.07	.12	-.06, .22	.09	.07	.15	-.05, .24	.13	.07	.21	-.01, .28
Afternoon Cortisol					-.27	.34	-.09	-.96, .41	-.06	.35	-.02	-.76, .64
Peer Acceptance									-.81	.39	-.25	-1.58, -.04
$\Delta R^2$	.02				.007				.05*			
$R^2$	.02				.03				.07			
Aroian test	$z = -1.61$											

Note:  $N = 88$ ; \*  $p < .05$  ; CI = 95% Confidence Intervals Lower, Upper

Table D10. Multiple regression analysis of afternoon cortisol on the mediator, teacher closeness (path *a*).

	Teacher Closeness							
	Step 1				Step 2			
	<i>B</i>	<i>SE B</i>	$\beta$	<i>CI</i>	<i>B</i>	<i>SE B</i>	$\beta$	<i>CI</i>
Sex	.55	.16	.34	.23, .86	.48	.15	.30	.18, .78
Age	.31	.13	.24	.06, .56	.20	.12	.16	-.05, .45
Afternoon Cortisol					1.97	.60	.32	.77, 3.17
$\Delta R^2$	.18***				.09**			
$R^2$	.18				.28			

Note: *N* = 89; \*\* *p* < .01; \*\*\* *p* < .001; *CI* = 95% Confidence Intervals Lower, Upper

Table D11. Multiple regression analyses testing the mediating effect of teacher closeness on the relations between afternoon cortisol and teacher-reported behaviour (path  $c'$ ), controlling for sex and age: Prosocial behaviour

	Teacher-rated Prosocial behaviour											
	Step 1				Step 2				Step 3			
	<i>B</i>	<i>SE B</i>	$\beta$	<i>CI</i>	<i>B</i>	<i>SE B</i>	$\beta$	<i>CI</i>	<i>B</i>	<i>SE B</i>	$\beta$	<i>CI</i>
Sex	.33	.11	.30	.12, .54	.26	.10	.25	.07, .45	.09	.08	.08	-.08, .26
Age	.24	.08	.27	.07, .40	.14	.08	.16	-.02, .29	.06	.07	.07	-.07, .20
Afternoon Cortisol					1.79	.38	.43	1.02, 2.55	1.07	.34	.26	.40, 1.73
Teacher Closeness									.37	.06	.55	.25, .48
$\Delta R^2$	.18***				.17***				.22***			
$R^2$	.18				.35				.56			
Aroian test	$z = 2.89^{**}$											

Note:  $N = 89$ ; \*\*  $p < .01$ ; \*\*\*  $p < .001$ ; *CI* = 95% Confidence Intervals Lower, Upper

Table D12. Multiple regression analyses testing the mediating effect of teacher closeness on the relations between afternoon cortisol and teacher-reported behaviour (path  $c'$ ), controlling for sex and age: Reactive aggression

	Teacher-rated Reactive Aggression											
	Step 1				Step 2				Step 3			
	<i>B</i>	<i>SE B</i>	$\beta$	<i>CI</i>	<i>B</i>	<i>SE B</i>	$\beta$	<i>CI</i>	<i>B</i>	<i>SE B</i>	$\beta$	<i>CI</i>
Sex	-.24	.12	-.20	-.48, .01	-.19	.12	-.16	-.42, .05	-.20	.13	-.17	-.45, .05
Age	-.09	.10	-.09	-.28, .11	-.01	.10	-.01	-.21, .18	-.02	.10	-.02	-.22, .18
Afternoon Cortisol					-1.35	.48	-.30	-2.29, -.40	-1.40	.51	-.31	-2.41, -.39
Teacher Closeness									.03	.09	.04	-.15, .20
$\Delta R^2$	.05				.08**				.001			
$R^2$	.05				.13				.13			
Aroian test	$z = .29$											

Note:  $N = 89$ ; \*\*  $p < .01$ ; *CI* = 95% Confidence Intervals Lower, Upper

Table D13. Multiple regression analyses testing the mediating effect of teacher closeness on the relations between afternoon cortisol and teacher reported behaviour (path  $c'$ ), controlling for sex and age: Proactive aggression

	Teacher-rated Proactive Aggression											
	Step 1				Step 2				Step 3			
	<i>B</i>	<i>SE B</i>	$\beta$	<i>CI</i>	<i>B</i>	<i>SE B</i>	$\beta$	<i>CI</i>	<i>B</i>	<i>SE B</i>	$\beta$	<i>CI</i>
Sex	-.02	.02	-.16	-.06, .01	-.02	.02	-.13	-.05, .01	-.01	.02	-.04	-.04, .03
Age	.00	.01	-.00	-.03, .03	.01	.01	.05	-.02, .03	.01	.01	.10	-.01, .04
Afternoon Cortisol					-.12	.06	-.21	-.25, .01	-.06	.07	-.11	-.20, .07
Teacher Closeness									-.03	.01	-.32	-.05, -.01
$\Delta R^2$	.03				.04#				.08**			
$R^2$	.03				.07				.14			
Aroian test	$z = -2.04^*$											

Note:  $N = 89$ ; #  $p = .059$ ; \*  $p < .05$ ; \*\*  $p < .01$ ; *CI* = 95% Confidence Intervals Lower, Upper

Table D14. Multiple regression analyses testing the mediating effect of teacher closeness on the relations between afternoon cortisol and teacher reported behaviour (path  $c'$ ), controlling for sex and age: Social aggression

	Teacher-rated Social Aggression											
	Step 1				Step 2				Step 3			
	<i>B</i>	<i>SE B</i>	$\beta$	<i>CI</i>	<i>B</i>	<i>SE B</i>	$\beta$	<i>CI</i>	<i>B</i>	<i>SE B</i>	$\beta$	<i>CI</i>
Sex	.04	.08	.05	-.13, .21	.05	.09	.07	-.12, .22	.05	.09	.06	-.13, .23
Age	.09	.07	.15	-.04, .23	.11	.07	.17	-.03, .25	.11	.07	.17	-.04, .25
Afternoon Cortisol					-.29	.34	-.09	-.97, .40	-.29	.37	-.10	-1.02, .43
Teacher Closeness									.005	.06	.01	-.12, .13
$\Delta R^2$	.03				.01				.000			
$R^2$	.03				.03				.03			
Aroian test	$z = .08$											

Note:  $N = 89$ ; \*  $p < .05$ ; \*\*  $p < .01$ ; \*\*\*  $p \leq .001$ ; *CI* = 95% Confidence Intervals Lower, Upper

Table D15. Multiple regression analyses testing the mediating effect of teacher closeness on the relations between afternoon cortisol and peer-nominated behaviour (path  $c'$ ), controlling for sex and age: Prosocial behaviour

	Peer-nominated Prosocial Behaviour											
	Step 1				Step 2				Step 3			
	<i>B</i>	<i>SE B</i>	$\beta$	<i>CI</i>	<i>B</i>	<i>SE B</i>	$\beta$	<i>CI</i>	<i>B</i>	<i>SE B</i>	$\beta$	<i>CI</i>
Sex	.15	.03	.42	.08, .22	.14	.03	.39	.07, .21	.11	.03	3.24	.04, .17
Age	.09	.03	.29	.03, .14	.07	.03	.24	.01, .13	.06	.03	2.12	.00, .11
Afternoon Cortisol					.28	.13	.20	.01, .54	.14	.13	1.03	-.13, .40
Teacher Closeness									.07	.002	.31	.03, .11
$\Delta R^2$	.27***				.04*				.07**			
$R^2$	.27				.31				.38			
Aroian test	$z = 2.17^*$											

Note:  $N = 88$ ; \*  $p < .05$ ; \*\*  $p < .01$ ; \*\*\*  $p \leq .001$ ; *CI* = 95% Confidence Intervals Lower, Upper

Table D16. Multiple regression analyses testing the mediating effect of teacher closeness on the relations between afternoon cortisol and peer-nominated behaviour (path  $c'$ ), controlling for sex and age: Reactive aggression

	Peer-nominated Reactive Aggression											
	Step 1				Step 2				Step 3			
	<i>B</i>	<i>SE B</i>	$\beta$	<i>CI</i>	<i>B</i>	<i>SE B</i>	$\beta$	<i>CI</i>	<i>B</i>	<i>SE B</i>	$\beta$	<i>CI</i>
Sex	-.10	.04	-.25	-.17, -.02	-.08	.04	-.21	-.15, -.01	-.08	.04	-.22	-.16, -.09
Age	-.12	.03	-.37	-.18, -.06	-.09	.03	-.29	-.15, -.03	-.09	.03	-.29	-.15, -.03
Afternoon Cortisol					-.47	.14	-.32	-.76, -.19	-.48	.15	-.32	-.79, -.18
Teacher Closeness									.005	.03	.02	-.05, .06
$\Delta R^2$	.21***				.09***				.00			
$R^2$	.21				.30				.30			
Aroian test	$z = .18$											

Note:  $N = 87$ ; \*  $p < .05$ ; \*\*  $p < .01$ ; \*\*\*  $p \leq .001$ ; CI = 95% Confidence Intervals Lower, Upper

Table D17. Multiple regression analyses testing the mediating effect of teacher closeness on the relations between afternoon cortisol and peer-nominated behaviour (path  $c'$ ), controlling for sex and age: Proactive aggression

	Peer-nominated Proactive Aggression											
	Step 1				Step 2				Step 3			
	<i>B</i>	<i>SE B</i>	$\beta$	<i>CI</i>	<i>B</i>	<i>SE B</i>	$\beta$	<i>CI</i>	<i>B</i>	<i>SE B</i>	$\beta$	<i>CI</i>
Sex	-.10	.04	-.29	-.17, -.03	-.09	.04	-.24	-.16, -.02	-.07	.04	-.21	-.15, .00
Age	-.05	.03	-.18	-.11, .01	-.03	.03	-.10	-.09, .03	-.03	.03	-.08	-.08, .04
Afternoon Cortisol					-.44	.14	-.32	-.71, -.16	-.38	.15	-.28	-.68, -.09
Teacher Closeness									-.03	.03	-.12	-.08, .02
$\Delta R^2$	.12				.09				.01			
$R^2$	.12				.21				.22			
Aroian test	$z = -.95$											

Note:  $N = 88$ ; \*  $p < .05$ ; \*\*  $p < .01$ ; \*\*\*  $p \leq .001$ ; CI = 95% Confidence Intervals Lower, Upper

Table D18. Multiple regression analyses testing the mediating effect of teacher closeness on the relations between afternoon cortisol and peer-nominated behaviour (path  $c'$ ), controlling for sex and age: Social aggression

	Peer-nominated Social Aggression											
	Step 1				Step 2				Step 3			
	<i>B</i>	<i>SE B</i>	$\beta$	<i>CI</i>	<i>B</i>	<i>SE B</i>	$\beta$	<i>CI</i>	<i>B</i>	<i>SE B</i>	$\beta$	<i>CI</i>
Sex	.04	.04	.10	-.04, .11	.05	.04	.15	-.02, .12	.05	.04	.16	-.02, .13
Age	-.04	.03	-.14	-.10, .02	-.01	.03	-.05	-.07, .05	-.01	.03	-.04	-.07, .05
Afternoon Cortisol					-.43	.14	-.33	-.71, -.15	-.41	.15	-.32	-.71, -.12
Teacher Closeness									-.01	.03	-.05	-.06, .04
$\Delta R^2$	.03				.10**				.002			
$R^2$	.03				.13				.13			
Aroian test	$z = -.38$											

Note:  $N = 87$ ; \*  $p < .05$ ; \*\*  $p < .01$ ; \*\*\*  $p \leq .001$ ; *CI* = 95% Confidence Intervals Lower, Upper

Table D19. Multiple mediation of the indirect effects of behaviour on afternoon cortisol through changes in peer acceptance and teacher closeness.

Behaviour	Multiple Mediator	<i>Point Estimate</i> (95% <i>BCaCI</i> )	Contrast (95% <i>BCaCI</i> )
<b>Peer Prosocial</b>			
Peer Acceptance	No	.16	-.009, .39
Teacher Closeness	Yes	.08	.02, .18
<b>Teacher Proactive aggression</b>			
Peer Acceptance	No	-.10	-.34, .001
Teacher Closeness	Yes	-.11	-.24, .21
<b>Teacher Social aggression</b>			
Peer Acceptance	Yes	-.02	-.07, -.002
Teacher Closeness	No	.002	-.03, .03

Note:  $N = 89$  for teacher-reported behaviours;  $N = 88$  for peer-reported behaviours.  
*BCaCI*= bias corrected bootstrapping samples and confidence intervals that include corrections for both median bias and skew. Confidence intervals containing zero are interpreted as not significant. A positive point estimate means an increase in ratings of behaviour as afternoon cortisol increases. Yes = A statistically significant mediation effect was observed.