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Individual differences in cortisol functioning: Longitudinal prediction of the relationship between psychosocial and physiological well being in mothers and children.

Dahlia Ben-Dat

A Thesis

in

The Department

of

Psychology

Presented in Partial Fulfillment of the Requirements for the Degree of Masters of Arts at Concordia University

Montreal, Quebec, Canada

July 2002

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ABSTRACT

Individual differences in cortisol functioning: Longitudinal prediction of the relationship between psychosocial and physiological well being in mothers and children.

Dahlia Ben-Dat

The purpose of this research was to explore the effects of an adverse psychological environment on physiological well being of mothers and their young children, within an ongoing inter-generational longitudinal project (Concordia Longitudinal Risk Project). The present research examined individual differences in diurnal cortisol functioning as predicted by a variety of historical and current psychosocial variables. Factors related to increased stress (e.g. maternal hostility, smoking, difficult temperament) were expected to predict deviations from the "normal" diurnal cortisol pattern in both mothers and children, whereas variables hypothesized to serve a protective or supportive function were expected to buffer these adverse effects. Salivary cortisol measurements were collected every two hours across one waking day in a sample of 40 mothers and their children, aged 2-6 years. Hierarchical linear modeling (HLM) growth curve analyses were used to estimate the intercept (morning cortisol level), and the slope (steepness of decline in cortisol values) for each mother and child's cortisol pattern across the daytime hours. Findings replicated the well-established diurnal cortisol pattern with high morning cortisol values that decline across the waking day. Consistent with the main hypothesis, a number of stress-related psychosocial factors were predictive of dysregulated cortisol functioning, including hostility, and child extraversion in mothers, and maternal withdrawal and smoking in their children. Other factors such as high SES, maternal

stimulation, and high child I.Q. appeared to serve a protective function. This longitudinal study illustrates the potential vulnerability associated with children raised in adverse circumstances and highlights the important relationship between psychosocial variables and physiological well being.

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Introduction

Adrenocortical functioning, part of the body's neuroendocrine response to stress, has been the focus of a great number of research studies. The role of the hypothalamic-pituitary-adrenal (HPA) axis, and the secretion of cortisol, has been implicated, in part, as a potential mechanism underlying the emergence of many negative outcomes in both adults and children (e.g. Chrousos & Gold, 1992; Granger et al., 1998; Heim et al., 2000). This hypothesis has led to the development of data-collection techniques and powerful statistical analyses to facilitate the inquiry into individual differences in cortisol patterns, and the negative correlates of dysregulated adrenocortical functioning.

More recently, researchers have begun to illustrate associations between diverse psychosocial variables, adverse environments, and deviations from typical cortisol functioning in healthy samples (for example: Ockenfels, 1995; Adam & Gunnar, 2001). As well, research has demonstrated the effects of unfavourable maternal psychosocial variables on the development of a child's adrenocortical functioning; many behavioural and physiological problems have been associated with such adrenocortical disturbance (Coplan et al., 1996; Susman et al., 2001; Cicchetti & Rogosch, 2001).

Cortisol research has yielded a muddy clinical picture in that dysregulation in cortisol patterns often take various forms. Whereas in the past, cortisol dysregulation typically referred only to elevated cortisol levels, there has been a recent emergence in the literature of hypocortisolism (i.e. low cortisol levels or flattened cortisol patterns) in relation to early adverse experiences and/or psychopathology (Heim et al., 2000; Gunnar & Vazquez, 2001). Longitudinal, developmental studies are necessary to verify whether

disrupted cortisol patterns in healthy people might mediate between adverse psychosocial factors and negative health outcomes.

Cortisol Functioning

Over the last several years, researchers have begun to study basal levels of the adrenocortical hormone, cortisol, as well as cortisol reactivity in response to stress, environmental events and social interactions (Caplan et al., 1979; Ockenfels et al., 1995; Smyth et al., 1997; Granger et al., 1998; Lupien et al., 2001). This work has led to a preliminary understanding of the manner in which cortisol functions. It has been established that cortisol is a steroid hormone that is secreted by the adrenal cortex during times of stress, and is regulated by the hypothalamic-pituitary-adrenal (HPA) axis. The secretion of cortisol leads to a chain of physiological events that prepares individuals to deal with stressors, and provides the brain and muscles with enhanced energy sources. Cortisol is a labile hormone that reacts to all stressful activities, including traumatic events, illness, surgery, drastic temperature change, exercise, strong feelings or emotions. and even daily activities such as eating. Cortisol is considered to be a reliable indicator of stress, such that many researchers define stress as an event that elicits increased levels of cortisol (Vander, et al., 1994). Non-invasive and reliable techniques of cortisol measurement through saliva samples are now commonly used, and facilitate the collection of repeated cortisol measures in naturalistic environments (Kirschbaum, 1994; Kirschbaum & Hellhammer, 1989).

Cortisol level is not however, simply a marker of stress. Some cortisol is present in the body at all times; it is necessary for the body to function in general. Thus, excesses or deficiencies in cortisol, as well as dysregulation in normal cortisol patterns have

negative consequences, and can lead to a number of physical symptoms and illnesses. Cortisol dysregulation can be present in many forms, including blunted cortisol reactivity to stress, flat cortisol levels across the day, or any other deviation from the typical diurnal pattern of cortisol secretion (Stone et al., 2001). Diverse patterns of cortisol dysregulation accompany various negative health outcomes (Heim et al., 1997). Correlates of disrupted cortisol function have been examined with respect to many psychiatric and physiological illnesses including depression, post-traumatic stress disorder (PTSD), eating disorders, hypertension, sexual dysfunction, and others (Cicchetti & Walker, 2001). Heim and colleagues (1997) provided an overview of the different effects of both acute and chronic stress on the HPA axis. In their review, they related specific and contradictory patterns of HPA dysfunction to symptoms of depression and PTSD. Researchers are currently investigating the relationship between disturbances in cortisol secretion and major depression in women (for example, Harris et al., 2000; Goodyer, Herbert, Tamplin, & Altham, 2000). In 2000, Heim and colleagues attempted to determine the relationship between hypocortisolism and early trauma, PTSD, chronic fatigue syndrome, fibromyalgia, other somatoform disorders, rheumatoid arthritis, asthma, and chronic stress.

Many studies have identified cortisol dysregulation in children with a history of maltreatment, depressive symptoms, and those reared in neglectful and abusive institutional settings (Kaufman, 1991; Hart, Gunnar, & Cicchetti, 1996; Gunnar, Morison, Chisholm, & Schuder, 2001; Carlson & Earls, 1997). Cicchetti and Rogosch (2001) found diverse patterns associated with different subtypes of maltreatment; for example, physical and sexual abuse, as well as neglect or emotional maltreatment was associated

with elevated cortisol levels, and a subgroup of physical abuse was associated with lower morning cortisol levels. Lupien and colleagues (2000; 2001) found higher cortisol levels in children from low socio-economic status (SES) backgrounds, with findings emerging as early as 6 years old. Finally, Granger and colleagues (1998) conducted research stemming from the same longitudinal investigation as the present study. The authors measured cortisol levels in 62 mother-child dyads before and after a conflict-oriented mother-child task. The findings demonstrated negative associations between pre-task cortisol levels and internalizing behaviour problems, maternal childhood levels of social withdrawal, and current psychosocial problems (Granger et al., 1988). The results of these studies provide clear evidence of the existence of an important relationship between psychosocial variables and adrenocortical functioning, which in turn, can be linked to physiological well being.

In the absence of external stressful events, cortisol secretion follows a robust diurnal pattern that is apparent in most healthy individuals. According to this pattern, levels of cortisol peak shortly after waking (approximately one half hour) and descend rapidly over the next few hours, followed by a more gradual and steady decline throughout the rest of the day to near-zero values at night before sleep. Cortisol levels show small rises and falls during the afternoon in response to hunger, satiety, and other natural events. (Weitzman. 1982; Schmidt Reinwald et al., 1999; Stone et al., 2001). This diurnal pattern is established early in life, first emerging at about 3 months of age (Price et al., 1983). A marked "normal" diurnal curve with high morning cortisol values (intercept) and a steep declining slope across the day are considered to be an indication of a healthy, reactive stress system (e.g. Heim et al., 2000; Adam & Gunnar, 2001).

Deviations from this robust diurnal pattern of HPA function, and specifically of cortisol secretion, have often been considered a cause for alarm, due to its association with psychopathology (Chrousos & Gold, 1992; Heim et al., 1997).

Caplan and colleagues (1979) have suggested that extremely weak, inconsistent or absent diurnal cortisol patterns are likely a sign of HPA dysregulation. Despite the strong diurnal pattern that cortisol displays, certain "healthy" individuals have cortisol patterns that deviate from the normal rhythm, and in some cases, even show a flat curve (Smyth et al., 1997; Stone et al., 2001). Further research has begun to examine whether more subtle individual differences in diurnal cortisol rhythms have psychological, physiological, or clinical significance (Adam & Gunnar, 2001). Researchers have recently begun to look at variables that predict individual differences in diurnal cortisol functioning in non-clinical populations. These studies have provided mixed results. For example, chronic stress as determined by unemployment has been found to relate to altered cortisol patterns (Ockenfels, Porter, Smyth, & Kirschbaum, 1995). Contrary to this work, however, Smyth and colleagues (1997) found that people with flat diurnal cortisol cycles did not significantly differ from people with normal or inconsistent cycles on a variety of demographic and psychological variables.

Adam and Gunnar (2001) examined individual differences in diurnal cortisol patterns in mothers of 2-year-old children as a function of demographic and medical variables, maternal relationship functioning, and home and work demands. They found a link between diurnal cortisol patterns and their measures of psychological well being. Specifically they found that women who report more positive feelings about relationships and effectively use them for support and comfort had higher morning cortisol values and

steeper slopes. This study provided evidence that systematic associations exist between certain psychosocial variables (the quality of relationships) and individual variability in physiological regulation of adrenocortical activity.

Animal models have been used to study the factors that lead to dysregulated HPA functioning. For example, Coplan and colleagues (1996) have studied the effects of adverse early experience on HPA activity in infant bonnet macaques. These monkeys were divided into three randomly assigned rearing conditions according to maternal foraging demands. The three groups included consistently low foraging demand (LFD), consistently high, but predictable foraging demand (HFD), and variable, or unpredictable foraging demand (VFD). The VFD group, and not the HFD group, was considered to be the most stressful condition simply because the unpredictable nature of the foraging demand caused mother macaques to experience a considerable amount of stress with regard to foraging expectations.

The authors found that as a result of the stressful, adverse environment, the VFD mothers displayed inconsistent, erratic, and sometimes dismissive rearing behaviour towards their children, possibly affecting the nature of the child's attachment to his or her mother. The mothers in this group demonstrated more anxiety and affective traits. In turn, the authors determined that infants reared in this condition displayed HPA dysregulation and exhibited increased affective vulnerability to the challenges of novel environments and maternal separation, behavioural inhibition, and less social behaviour. These dysregulations in HPA functioning were sustained 30 months following the termination of the manipulation of the conditions of maternal foraging demand (Coplan et al., 2001). These findings support the hypothesis that stressful childhood environments can produce

long-lasting disturbances of the HPA axis, making individuals more vulnerable to psychological disorders as adults. The authors highlighted the importance of a secure mother-infant relationship during a critical development period with regard to health outcome. They demonstrate a link between maternal psychosocial stress and well being in children.

The question remains whether research similar to Coplan's work will demonstrate associations between maternal stress, compromised attachment relations, and an adverse rearing environment with disrupted cortisol functioning in children. In turn, should this association exist, might it be an indication or possibly even a precursor of future behavioural disturbances and negative health outcomes?

Few studies have demonstrated the link between maternal stress and negative outcomes in children. Susman and colleagues (2002) revealed that negative child temperament, as characterized by verbal and nonverbal aggression at age 3, was associated with maternal stress as measured by low levels of maternal prenatal hormones, including cortisol. More studies are required to elucidate the role that adrenocortical functioning plays in mediating between an adverse psychosocial environment and a variety of medical and psychological problems. We must investigate the causes of disruptions in diurnal rhythms of cortisol, and study the negative correlates of such dysregulation. This knowledge could potentially lead to the prevention of a variety of negative health outcomes. Therefore, an examination of children's cortisol functioning as predicted by maternal psychosocial variables is warranted.

Questions Remaining to be Answered/Issues investigated in this study

The participants in the present exploratory study were 40 mothers and 40 children drawn from the Concordia Longitudinal Risk Project (CLRP). The CLRP was initiated in 1976 to examine atypical childhood behavior, such as high levels of aggression and social withdrawal, as predictors of adult psychopathology. This data set provides considerable information about the mothers of the children involved in the present study. Data collection has been ongoing since 1976, and children have been studied from their date of birth. Information is available concerning historical, demographic, behavioural, temperamental, and health variables that could be predictors of individual differences in cortisol functioning. No previous study has been conducted to assess individual differences in diurnal cortisol functioning within the context of such a longitudinal study. No studies to date have examined the diurnal cortisol functioning of children, as predicted by a set of maternal variables that match the number and scope of the variables examined in this study.

This present study was conducted to examine the associations between a large number of psychosocial variables and the patterns of cortisol functioning across the waking day of mothers and their children. Hierarchical Linear Modeling (HLM) growth curve analyses techniques (Bryk & Raudenbush, 1992) were used to model and predict individual differences in diurnal cortisol patterns. HLM provides a number of advantages for the analysis of nested data such as the repeated cortisol samples collected in this study. For example, individuals are not required to sample at exactly the same time of day because they are not compared on individual cortisol samples, but rather on the line of best fit through all of their samples (Adam & Gunnar, 2001). For this reason, missing

data points are not as critical, provided a valid slope can be applied to the data for each individual. HLM allows for a simultaneous analysis of multiple levels of information for each individual; thus, it provides an efficient way to account for the effects of time of day, as well as a number of specified predictor variables, on individual growth curves of cortisol functioning. Only one study to date has analyzed similar data according to HLM (Adam & Gunnar, 2001).

Hypotheses/Predictions

It was hypothesized that strong diurnal cortisol cycles would be detected in both mother and child samples, peaking just after waking, and declining across the day. Second, there would be individual variability in both the intercept and slope of diurnal cortisol in both mother and child samples. The third hypothesis was that for both mother and child samples, variables that could be considered positive such as high SES, positive maternal stimulation, and high child I.Q. would have a protective effect on the "normal" strong diurnal cortisol pattern; whereas variables that can be considered negative, such as maternal hostility, maternal childhood social withdrawal, difficult child temperament, and maternal smoking would influence diurnal cortisol in a detrimental manner, either by changing the morning cortisol intercept, or by altering the slope such that cortisol levels do not decline normally across the day.

Method

Participants

The participants in this study were 40 mothers and 40 children drawn from a larger, longitudinal study, the Concordia Longitudinal Risk Project (CLRP). For a description of the original longitudinal study, see Schwartzman et al., (1985). Four children were removed from the analyses due to insufficient cortisol data.

The women in the present sample were Caucasian and francophone, living in and around the Montreal area, and ranged in age from 26.16 to 33.92 years (M=31.23, SD = 2.34). The range of mothers' ages at the time of birth of their first child was 19.91-30.81 (M=26.63; SD=2.93), and the number of children per mother ranged from 1-6, with the mean, mode, and median being 2 children (SD=.959). Thirty-five of the women were married, and 5 were single. The mothers had between 5 and 18 years of school (M=12.08, SD=2.69). In Quebec, a high school-graduate is considered to have eleven years of education. Nine (22.5%) women in this sample did not complete high school.

Mother's occupational prestige was rated according to a scale devised by Nock and Rossi (Rossi et al., 1974). Their ratings ranged from 162 to 589 (M=343.10, SD =114.57), corresponding to jobs ranging from maids and cleaners, to office managers and schoolteachers. The mean value (343) corresponded to jobs such as tailors, mechanics, sales clerks, and flight attendants. These women had a mean yearly family income of \$44,587, with a median of \$41,704 (SD = \$25,581; range = \$8,430-\$127,982). Fourteen women (35%) in this sample had incomes that were below the poverty line, including 4 (10%) who were receiving social assistance. Ratings of marital status, family income, and

family occupational prestige were aggregated into a socio-demographic variable that was used in analyses.

The 36 children in this sample ranged in age from 1.91 to 6.12 years (M=4.82, SD=1.09), although the majority of children were above 4 years old (n=27; 75%). There were 17 boys and 15 girls. Their level of IQ based on the Stanford-Binet-IV I.Q. test (n=33) ranged from 85-119 (M=100.91; SD=8.983). Table 1 presents a summary of the demographic characteristics of the current sample.

Procedure

Two visits were made to the participants' homes, lasting approximately 3 hours each. Participants were informed of the general nature and procedures of the study, but not of the specific research hypotheses. The research team consisted of a licensed, M.A. level psychologist and a research assistant.

The visits included an intellectual assessment, naturalistic observations, interviews, and questionnaires. At the beginning of the first session, the psychologist outlined the procedure to the mother and asked her to read and sign an informed consent form (see Appendix A). Either the Bayley Scales of Infant Development (Second Edition: Bayley, 1993) or the Stanford-Binet Intelligence Scale (Fourth Edition; Thorndike, Hagan, & Sattler, 1986) was administered, depending on the child's age, to assess current intellectual functioning. The mothers completed interviews and a variety of questionnaires assessing their own and their child's physical health, behaviour, and temperament. Structured interaction sessions between mother and child were conducted and were videotaped in order to provide observational measures of temperament,

Table 1

Means, Standard Deviations, Medians and Ranges of Demographic Information

(Mother N=40; Child N=36)

	Mean (SD)	Median	Range
Mother's Current Age	31.23 (2.34)	32.22	26.16 - 33.92
Mother's age at first child	26.63 (2.93)	27.26	19.91-30.81
Number of children	2.05 (0.959)	2.00	1.00-6.00
Maternal Education	12.08 (2.69)	12.00	5.00 - 18.00
Occupational Prestige*	343.10 (114.57)	314	162 – 589
Family Income	\$44,587 (\$26,581)	\$41,704	\$8,430-\$127,982
Child's Current Age	4.82 (1.09)	5.23	1.91 - 6.12
Child's I.Q. (based on the SB-IV)	100.91 (8.983)	102.50	85-119

^{*} Nock & Rossi Scale of Occupational Prestige (Rossi et al., 1974).

maternal stimulation, and other mother-child interaction variables. These interactions included semi-structured free play sessions and a structured puzzle task.

At the end of the second visit, participants were left with a request for cortisol sampling, instructions on how to complete the protocol, instruments or materials necessary for such sampling, and a return envelope. Further details of the method of cortisol sampling are provided below. A complete description of the protocol for both home visits can be found in Appendix B. Further descriptions of the play sessions and the puzzle task can be found in Karp (2000), and Saltaris and Samaha (1998) respectively.

Cortisol sampling

Mothers and their children were requested to collect saliva cortisol samples upon waking in the morning, and again every two hours until participants went to sleep at night. For a full description of the cortisol protocol, please see Appendix C. The method of cortisol sampling was an adaptation of that used by Stahl and Dorner (1982). Subjects were asked to hold a strip of filter paper (65mm x 25mm, Watman 1) under their tongue until it was saturated with saliva. Saliva samples were to be air dried, and frozen at minus 20°C until they were assayed for cortisol. The assays were performed at the Douglas Hospital Research Laboratories (DHRL). Cortisol levels were determined via competitive protein binding radioimmunoassay, using a radioimmunoassay procedure developed by Krey et al., (1975). Variability of intra- and inter-assay reliability and validity coefficients had been determined in previous studies at the DHRL and were well within acceptable limits (range: 3.5-5.0%). Sensitivity unique to saliva cortisol was high (Laudat et al., 1988). Cortisol antibody (F3314) was obtained from Endocrine Sciences, CA, and

other [3H] cortisol was purchased from New England Nuclear, Boston, MA to serve as the tracer.

Measures

When French versions of measures used in this study were not available, English measures were translated into French by a research analyst for use with this Francophone sample according to reliable methods (Senneville, 2002).

Demographic Information

In order to obtain socio-demographic information on the families participating in the study, a Demographic Information Questionnaire (DIQ; see Appendix D) was used. From this questionnaire, information was obtained with respect to mother's current age, age at the birth of her first child, marital status, educational level, occupational status, current income, number and ages of children in her family. The DIQ was administered over the phone at the time that the home visit was being arranged.

Parental Aggression and Withdrawal

To examine childhood levels of aggression and withdrawal of the parents in this study, we drew from information that was collected as part of the original longitudinal study from which this sample was recruited. Between 1976 and 1978, when these parents were children, they were screened using a peer evaluation measure, the Pupil Evaluation Inventory (PEI; Pekarik et al., 1976; see Appendix E). Studies have shown that peer nominations represent a reliable method of rating children's behavior (Lyons, Serbin, & Marchessault, 1988). Peer evaluations have been found to be good predictors of problems of adjustment in children (Landau, Milich, & Whitten, 1984; Rolf, 1972). The PEI contains 35 items that load on three separate factors, aggression, withdrawal and

likeability. In the present study, the continuous dimensions of aggression and withdrawal were used as predictors of diurnal cortisol.

Temperament and Hostility

The temperamental characteristics of the children in this study were determined according to two methods. The first was according to videotaped mother-child interactions using the Behavioural Style Coding System (BSCS; Karp, 1999). In this coding system, child mood, activity level, reactivity, approach to toys, and mood regularity are each coded on 3-point scales. Inter-rater reliability for this coding system was high, with percent agreement ranging from 84% to 99%. A temperament factor based on each child's average mood, activity level, regularity, reactivity, approach to toys, and adaptability was used in analyses.

A second measure of temperament was obtained from the Emotionality Activity and Sociability Scale, 2nd version (EAS-2; Buss and Plomin, 1984)(Buss, 1989; Buss, 1991). For the purposes of this study, both The EAS Temperament Survey for Adults and The EAS Survey for Children: Parental Ratings were used. Both versions are self-report questionnaires with 20 statements that adults rate on a scale of 1-5 how characteristic or typical each item is of oneself, or in the latter case of one's child. In the adult version, the emotionality factor breaks down into 3 sub-factors: distress, fearfulness, and anger. In the EAS-2 for children, distress emerges as a sub-factor of emotionality, and shyness emerges as a sub-factor of sociability. Test-retest reliability measures of the adult survey are good and range from .75-.85, and similar psychometric properties exist for the child measure (Buss and Plomin, 1984).

In order to determine levels of maternal hostility, a second coding scheme, derived from the Emotional Availability Scale (EAS; Biringen, Z. & Robinson, J., 1988) (Biringen, 1988) was used to score the videotaped mother-child interactions. The Emotional Availability Scale has five subscales: maternal sensitivity, maternal structuring/intrusiveness, maternal overt and covert hostility, child responsiveness to mother, and child involvement with mother. Inter-rater reliability was assessed using intraclass correlation coefficients (Shrout & Fleiss, 1979), and r's ranging from .84 to .99 were obtained (Bentley, 1997).

Maternal Teaching

In order to assess the quality of maternal teaching style, including mothers' abilities to stimulate their children, each mother-child puzzle task interaction was rated using the Maternal Teaching Observation System (MTOS), as designed by Saltaris and Samaha, (1998). This coding system included both global and specific ratings of maternal teaching, capturing general qualities of maternal sensitivity and involvement in the task, as well as specific suggestions, strategies, and directions towards her child. Inter-rater reliability ranged between .62-.98. For a more detailed description of this coding scheme, please refer to Saltaris (1999).

Children's Cognitive Development

Depending on the child's age, one of two test batteries was administered to assess cognitive processes, verbal and motor expressive functions, as well as auditory and visual receptive functions. For children aged 12-42 months (N=3), the Bayley Scales of Infant Development (Second Edition, Bayley, 1993) was administered. The majority of children in this study, aged 43-72 months (N=33), were administered a French translation of the

Stanford-Binet Intelligence Scale (SB-IV; Thorndike, Hagen, & Sattler, 1986) (Thorndike, 1986).

Quality of the Home Environment

To measure the stimulation and support offered to the child in the home, The Home Observation for Measurement of the Environment (HOME; Caldwell & Bradley, 1984) was administered to each family. Depending on age, two versions of the HOME test were administered: the infant HOME, appropriate for children 0-3 years of age, and the preschool HOME, appropriate for children 3-6 years of age. A composite score is derived for the quality of the home environment by summing the number of yes responses across all items; the higher the score, the higher the quality of the home environment. The HOME inventory has demonstrated good psychometric properties (Luster & Dubow, 1992). In the current study, internal consistency was determined to be .77 for the infant version and .70 for the preschool version.

Social Support Satisfaction

A modified version of the Parenting Social Support Index (PSSI; Telleen, 1985) (Telleen, Herzog, & Kilbane, 1989) that was appropriate for parents of toddlers and preschoolers was used to assess parenting social support. The PSSI is a self-report measure with 22 items. Respondents are asked to rate their satisfaction with the support they receive on 5-point Likert scales. By summing across items, 3 composite scores are created including total perceived need for support, total network size, and total support satisfaction. The PSSI demonstrates good reliability and validity (Telleen, 1985). The 3 composite scores were used in analyses in this study.

Parenting Stress

The Parenting Stress Inventory (PSI-II; Abidin, 1986) was employed to determine the level of stress experienced by mothers in the current study. This self-report measure was designed to assess the sources and levels of stress perceived by individuals in relation to their parenting roles and responsibilities. Parents are asked to rate the degree to which each statement is true along a 5-point Likert scale. A total score indicating global parenting stress is computed from these items. The psychometric properties of the PSI are well documented. The reliability coefficients for the subscales of the PSI and the total score are .90 or above (Abidin, 1986).

<u>Analyses</u>

The data in the current study were analyzed using Hierarchical Linear Modeling (HLM), a technique developed by Bryk and Raudenbush (1992). Hierarchical linear models are widely used in behavioural and social data, which commonly have a nested structure. The authors explain that when repeated observations are collected on a set of individuals and the measurements are not identical for all people, the multiple observations are properly conceived as nested within persons. A unique sub-model represents each level in the data structure within the hierarchical linear model (e.g., repeated observations within persons, persons within communities, communities within states). Each sub-model represents the structural relationship that occurs at that level, along with the residual variability (Bryk & Raudenbush, 1992).

In the present study, the HLM/2L program (Bryk et al., 1996) was used to address the relationship between a number of psychosocial variables and individual differences in cortisol functioning in mothers and children. HLM growth curve analyses were used to

estimate the intercept (early morning level), and the slope (steepness of decline in cortisol values across the day) of each mother and child across the waking hours. HLM techniques were then used to predict individual differences in these parameters using the predictors of interest.

Results

Cortisol Collection

The mean number of saliva cortisol samples collected across the day, from the time of arousal to the time before going to sleep was 7.54 (SD=0.19; range 3-12). When broken down into mothers and children, the mean number of saliva cortisol samples collected across the day by mothers was 8.10 (SD=0.24; range 4-12) and the range of cortisol values was $0.10 \mu g/dl - 6.274 \mu g/dl$. The mean number of saliva cortisol samples collected across the day by children was 6.96 (SD=0.27; range 3-12), and the range of cortisol values was $0.10 \mu g/dl - 9.689 \mu g/dl$. It is important to understand that cortisol levels fluctuate with all activity, and thus some high values are reasonable when taking into consideration that each person's surroundings and emotionality were not controlled for in this study. Based on the average values of cortisol sampled at each 2-hour time period, the mothers' cortisol values ranged from 0.47 μ g/dl – 1.02 μ g/dl, and the children's values ranged from 0.39 μ g/dl - 0.85 μ g/dl. The repeated measures of cortisol that were collected ensure that analyses could be performed with many predictors, despite the small number of people in this sample. HLM treats each cortisol sample as a discrete measurement, nested within a person, who is in turn, nested within a mother-child dyad. The multiple cortisol samples per person increased the available degrees of freedom by a great deal, despite the relatively small sample size.

Diurnal Cortisol Curves

Typical daytime basal HPA activity involves high levels of cortisol in the morning, usually peaking around a half-hour after waking. This peak is followed by a rapid descent in cortisol over the next few hours, followed by a more gradual and steady

decline throughout the rest of the day to near-zero values at night before sleep. Smaller rises and falls of cortisol occur throughout the day in response to hunger as well as to other somewhat stressful events or activities. Figures 1 and 2 illustrate that the cortisol data for the participants in this study follow this basic, diurnal pattern. This confirms the first hypothesis: namely, that time heavily influenced the pattern of diurnal cortisol secretion in this sample, replicating previous findings (Price et al., 1983; Stone et al., 2001).

Hierarchical linear modeling procedures (HLM; Bryk and Raudenbush, 1992) were used to fit separate lines of best fit to the time-cortisol data of each participant. These techniques produced estimates of the morning value or intercept (B0), as well as the decline across the day, the slope (B1) of each individual participant's daily cortisol curve. HLM also provided an estimate of the average intercept and slope of the diurnal curves across all participants. HLM analyses were performed separately for the sample of mothers and the sample of children respectively. In both samples, the cortisol data were centered such that the intercept of the regression of time on cortisol represented the time between 8 a.m. and 10 a.m. In doing so, the data were analyzed from the point just after the peak, when cortisol levels began their decline across the day.

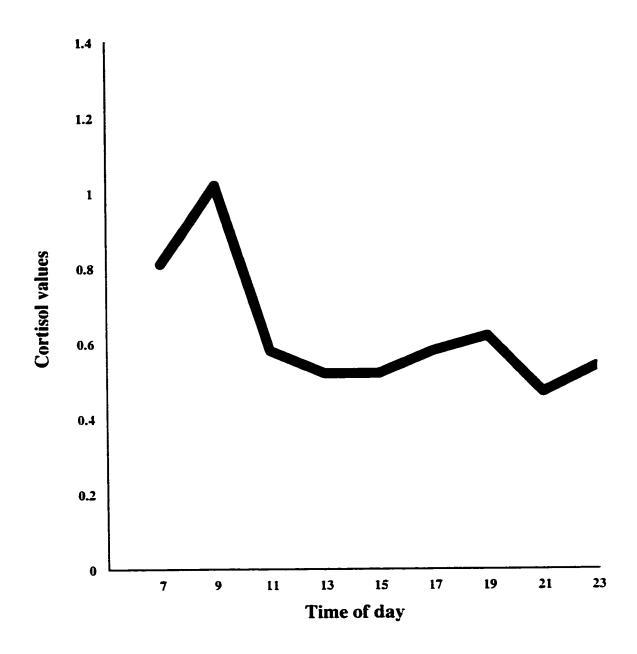


Figure 1. Average diurnal cortisol pattern of mothers.

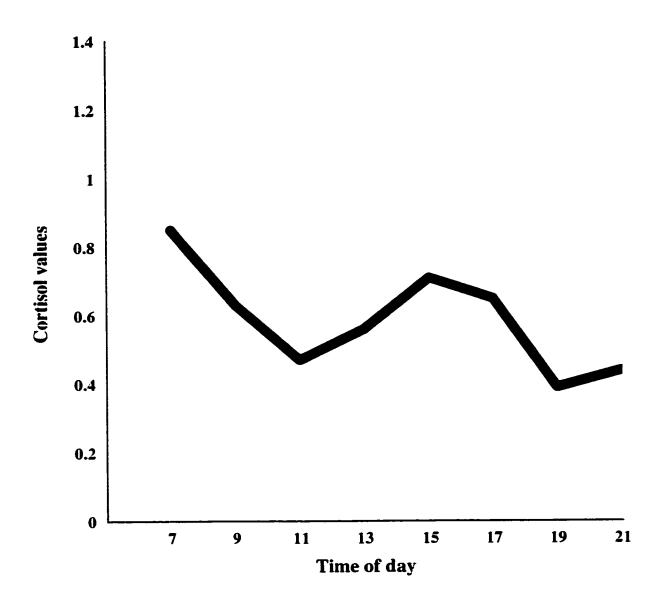


Figure 2. Average diurnal cortisol pattern of children.

Level 1 HLM Model

The HLM/2L program (Bryk et al., 1996) involves two levels. At the Level 1 HLM Model, a pattern of diurnal cortisol is described for each individual by estimating lines of best fit through the available data (cortisol values) based on the time-of-day of sampling. This is similar to regressing time-of-day onto cortisol. The obtained intercept and slope parameters of these lines (representing estimated early morning cortisol values, and the rate of change in cortisol levels across the day) can then be related to individual characteristics in the Level 2 HLM Model by inclusion of different predictor variables. Note that HLM uses an algorithm that takes into account the reliability of the data for each individual, weighting more reliable (better fitting) data more heavily, and does not simply calculate an average from individual intercept and slope values.

The Level 1 Models predicting cortisol intercept values from time of day in both the mother and child samples were highly significant (p≤ .000; see Tables 2 and 3 Fixed Effects). However, in both mother and child samples, the prediction of cortisol slope values from time of day were not significant, suggesting that the value of the slope does not differ significantly from zero. It is clear, however, that when looking at individual values, participants in the sample do, indeed, have cortisol slopes, and do not have flat curves. Reasons for this discrepancy might be that the values of different individuals' slopes cancel each other out, as some are positive and some negative, resulting in a net value close to zero.

A calculation of the intra-class correlation (a statistic that is calculated from the sigma squared and tau values) indicated that 48% of the observed variability in cortisol values was accounted for by time of day in the level 1 model in the mother sample. That

is, for the mothers, 52% of the variability in cortisol is unexplained by the Level 1 HLM Model. In the child sample, 60% of the observed variability in cortisol values was accounted for by time of day in the level 1 model, leaving 40% unexplained variance. All unexplained variance could be further examined in the Level 2 HLM Model. A significant amount of individual variation around these average slope and morning intercept values exists in both mother and children samples, and thus confirms the second hypothesis (see Tables 2 and 3, Random Effects). This is of great interest as it suggests that this variability might be explained by individual characteristics in a Level 2 HLM Model.

Level 2 HLM Model

Exploratory analyses were conducted in HLM to examine which individual variables might be important predictors of mothers and children's cortisol intercept and slope values. These exploratory analyses were based on working hypotheses of which psychological variables might influence cortisol functioning. Among the variables tested were age, sex, family income, poverty, prestige, marital status, cigarettes smoked by the mother, maternal education, maternal perceived stress variables from the PSI (Abidin, 1986), maternal social support (PSSI; Telleen, 1985), parental childhood ratings of aggression and withdrawal, temperament variables on both the mother and child based on the EAS (Buss and Plomin, 1984), observed temperament (BSCS, 1999), home environment (HOME, 1985), maternal stimulation (MTOS, 1999), child's IQ, and observational measures of maternal hostility, child involvement and responsiveness (EAS; Biringen & Robertson, 1988).

Potentially important predictors that had demonstrated statistical significance or trends toward significance in exploratory analyses were then entered simultaneously in a multivariate HLM model. With respect to demographic information, the age of mothers and children, and the child's sex played no role in predicting differences in cortisol curves and were dropped from the model. Maternal education, marital status, family income, and family prestige were all significantly related to the cortisol parameters. In order to simplify the level two model, marital status, family income, and prestige were standardized, and factor analysis resulted in one socio-demographic variable that was later used in the final model.

Maternal cigarette smoking predicted significantly lower cortisol intercepts in the children's sample, but not in mothers' sample. Contrary to expectations, the PSI, dealing with maternal perceived stress, and the PSSI, relating to social support satisfaction, did not demonstrate any significance with respect to the cortisol intercept and slope, and were removed from the final model. Neither time 1 withdrawal nor aggression influenced the mother's cortisol, however time 1 withdrawal did predict significant differences in cortisol intercepts and slopes in the children's sample, so only withdrawal was retained for final analyses. With respect to Buss & Plomin's EAS (1985), the sub-factors of child sociability and activity were significantly related to cortisol functioning. These sub-factors were aggregated into one variable termed child extraversion that was retained for the final model. Finally, all of the observational measures predicted significant individual differences in the intercepts and slopes of both mothers and children, and were therefore retained in the final model.

Results of the final multivariate HLM models for both the mother and child samples are presented in Tables 4 and 5 respectively. The coefficients reflect the independent contribution of each variable beyond the effects of the other variables in the model. In mothers, the socio-demographic factor variable, comprised of marital status, family income and family prestige, was a strong predictor of individual differences in both cortisol intercept and slope. Higher scores on the socio-demographic factor variable predicted somewhat higher cortisol intercepts (p<0.10), and steeper slopes (p<0.05). Higher maternal hostility predicted markedly lower intercepts (p<0.05) and more shallow slopes that differed significantly from the normal pattern and possibly inclined slightly (p<0.001). Finally, highly extraverted children, as measured by a factor score comprised of the sociability and activity scales of the EAS, predicted elevated cortisol intercepts (p<0.05), but had slopes that did not differ from the norm.

The variables that predicted significant differences in the children's cortisol parameters were different from those that predicted significant differences in the mother's sample. High ratings of maternal childhood social withdrawal predicted lower cortisol intercepts (p<0.05) and slopes that did not decline according to the normal pattern, but were shallow and possibly even inclined slightly (p<0.05). Mothers' smoking predicted very low cortisol intercepts (p<0.05), but smoking did not predict differences in children's cortisol slopes. Mothers' ability to stimulate her child predicted steeper, declining cortisol slopes (p<0.01). Finally, higher child I.Q. predicted steeper cortisol slopes than did lower I.Q.s (p<0.05). Thus, the third hypothesis was confirmed as predictors of individual differences in cortisol functioning affected the diurnal pattern in the anticipated direction (i.e. "negative" predictors were associated with deviations from

the "normal" diurnal curve characterized by relatively high intercepts and steep, declining slopes, while "positive" predictors appeared to buffer against these adverse effects). Predictor variables that significantly affected the cortisol intercepts and slopes did so in a generally consistent manner. "Negative" variables such as a lower score on the sociodemographic factor, high maternal hostility, high maternal childhood social withdrawal, and maternal smoking were associated with lower cortisol intercepts. Only child extraversion demonstrated contradictory results, with an elevated cortisol intercept. "Positive" variables such as a high score on the socio-demographic factor, good ability to stimulate your child, and high child I.Q. were all associated with a steeper, more marked slope across the day, whereas "negative" variables such as high hostility and high maternal childhood social withdrawal were associated with slopes that were shallow and possibly inclining rather than declining.

Table 2

Level 1 HLM Model: Mothers' cortisol values predicted by time of day (n=40)

Fixed Effect	Coefficient	SE	T-ratio	P-value
Average cortisol intercept	0.605	0.063	9.545	0.000
Average cortisol slope	-1.71 x 10 ⁻⁴	0.001	-1.609	0.115
Random Effect		Variance Component	Chi-square*	P-value
Cortisol		0.108	61.653	0.000
intercept Cortisol slope		0.0005	58.967	0.001

^{*} The chi-square statistics reported above are based on only 29 of 40 units that had sufficient data for computation. Fixed effects and variance components are based on all the data.

Table 3

Level 1 HLM Model: Children's cortisol values predicted by time of day (n=36)

Fixed Effect	Coefficient	SE	T-ratio	P-value
Average cortisol intercept	0.545	0.0939	5.802	0.000
Average cortisol slope	-4.7 x 10 ⁻⁵	1.6 x 10 ⁻⁴	-0.300	0.766
Random Effect		Variance Component	Chi-square*	P-value
Cortisol intercept		0.244	106.269	0.000
Cortisol slope		0.000	56.852	0.006

^{*} The chi-square statistics reported above are based on only 34 of 36 units that had sufficient data for computation. Fixed effects and variance components are based on all the data.

Table 4
Level 2 HLM Model: Multivariate model predicting mothers' morning cortisol values and slopes from selected variables (n=40)

Fixed Effect	Coefficient	SE	T-ratio	P-value
				
Predicting cortisol intercept				
Intercept	0.853052	0.444169	1.921	0.063+
Socio-demographic Factor	0.079003	0.046619	1.695	0.0 9 9 ⁺
Hostility	-0.383626	0.176971	-2.168	0.037*
Child Extraversion	0.113157	0.054303	2.084	0.044*
Mother's Education	0.012146	0.024105	0.504	0.617
Mother's Smoking	0.030699	0.163202	0.188	0.852
Predicting cortisol slope				
Intercept	0.002196	-0.000821	-2.674	0.012**
Socio-demographic Factor	-0.000312	0.000124	-2.507	0.017*
Hostility	0.001242	0.000331	3.749	0.001***
Child Extraversion	0.000070	0.000094	0.747	0.460
Mother's Education	0.000063	0.000048	1.309	0.200
Mother's Smoking	-0.000036	0.000218	-0.163	0.872
Random effect		Variance	Chi-square	P-value
		Component		
Cortisol intercept		0.09069	48.95148	0.002
Cortisol slope		0.00042	0.00000	0.007

*** p≤0.001; **p≤0.01; *p≤0.05; *p≤0.10

Table 5
Level 2 HLM Model: Multivariate model predicting children's' morning cortisol values and slopes from selected variables (n=36)

Fixed Effect	Coefficient	SE	T-ratio	P-value
Predicting cortisol intercept				
Intercept	0.523632	0.809478	0.647	0.523
Time 1 Withdrawal	-0.155598	0.078825	-1.974	0.058*
Socio-demographic Factor	-0.044274	0.062618	-0.707	0.485
Child Stimulation	0.122431	0.102045	1.200	0.241
Mother's Education	0.017600	0.050058	0.352	0.728
Child Temperament (obs.)	0.004402	0.084109	0.052	0.959
Child I.Q.	0.073304	0.102242	0.717	0.479
Child Sex	-0.077409	0.179219	-0.432	0.669
Mother's Smoking	-0.506016	0.195543	-2.588	0.016*
Predicting cortisol slope				
				0.560
Intercept	0.000764	0.001296	0.590	0.560
Time 1 Withdrawal	0.000402	0.000191	2.105	0.044*
Socio-demographic Factor	0.000051	0.000099	0.509	0.614
Child Stimulation	-0.000441	0.000154	-2.865	0.008**
Mother's Education	-0.000015	0.000082	-0.182	0.857
Child Temperament (obs.)	-0.000104	0.000132	-0.785	0.439
Child I.Q.	-0.000376	0.000182	-2.065	0.048*
Child Sex	-0.000238	0.000294	-0.808	0.426
Mother's Smoking	-0.000215	0.000334	-0.644	0.525
Random effect		Variance Component	Chi-square	P-value
Cortisol intercept		0.25593	73.03850	0.000
Cortisol slope		0.00067	38.74091	0.039

^{***} p≤0.001; **p≤0.01; *p≤0.05; *p≤0.10

Discussion

These results suggest systematic associations between a number of psychological variables and individual differences in patterns of diurnal cortisol across one waking day in a sample of 40 women and 36 children. First, we have replicated findings that a robust, diurnal pattern of cortisol exists, reaching highest levels following waking and declining across the waking day, with lowest levels at night. As well, these findings illustrated that individual variation exists among the people in the current sample as demonstrated by significant between-subject variability. Finally, a portion of this variability was explained in part by a number of psychosocial predictor variables including SES, maternal hostility, child extraversion, maternal childhood withdrawal, maternal smoking, maternal stimulation, and child I.Q.

Further research is necessary to elucidate the mechanisms by which such associations might emerge, and to better understand the clinical and health significance of these individual differences in diurnal cortisol cycles. On a surface level, these findings might best be understood according to the idea that variables that are associated with high levels of stress or disadvantage are related to dysregulation in diurnal cortisol. This can be illustrated in the mother sample, for example, by the association of disrupted cortisol with low SES and high maternal hostility. In the children's sample, high maternal withdrawal can be perceived as stressful for a child if his mother is unable to be an effective parent and a secure attachment figure. As well, poor parental ability to stimulate a child, along with low child I.Q. can cause additional stress and disadvantage to that child. Maternal smoking might be linked to a physiological mechanism that affects cortisol levels, although it is surprising that statistically significant associations only

emerged between maternal smoking and the children's diurnal cortisol, and not the mothers'. It is important to note that additional explanations for these associations are plausible, for example temperamental factors or genetic relationships might account for some of these findings. Further exploration into the mechanisms underlying such associations would provide a greater understanding of etiology.

The level 2 models accounted for a portion of explained variance above and beyond the factor of time (level 1 models) as demonstrated by the final estimation of variance components in both the mother and child models. The level 1 model accounts for within-group variance (i.e. the variance between individual cortisol samples within each individual curve). Level 1 accounted for the effect of time on the different cortisol samples. However, between-group variance remained to be explained above and beyond the effect of time. The level 2 model accounts for this between-group variance (i.e. the variance between each individual's cortisol growth curves). Level 2 attempts to account for this remaining between-group variance using predictor variables. In HLM, one may continue to attempt to account for this between-group variance until the p value ceases to be significant (p \geq .05), meaning there is no longer any remaining between-group variance to be explained, and the model has completely accounted for the variance by the predictor variables in the level 2 model. Therefore, one can see that the level 2 models have, in fact, accounted for more variance than the level 1 models, and this can be attributed to the predictor variables that demonstrated statistical significance in the two respective samples.

The intra-class correlation is a statistic that measures explained variance. It is calculated by a ratio of variance (measured by the tau statistic) over variance plus error

variance (measured by tau plus sigma squared). In the mother sample, the intra-class correlation for the level 2 model was 0.43, meaning that 43 % of the variance was explained by the model (time, SES, maternal hostility and child extraversion) while 57% was still unexplained. In the child sample, the intra-class correlation for the level 2 model was 0.62, meaning that 62% of the variance was explained by the model (time, maternal withdrawal, maternal smoking, maternal stimulation, child I.Q.) while 38% was left unexplained.

Despite the fact that a sizeable portion of the variance remains to be accounted for in both samples, and the unique contribution of each individual predictor is not very large, these results must be interpreted in the context of the current sample. The fact that any statistical significance emerged in this exploratory study, with 40 mothers and 36 children, speaks strongly for itself. In this pilot study, the small sample size limited the extent to which the results could be clinically significant. However, with a larger sample, the power and the effect sizes of the findings would be substantially larger, and based on an extrapolation of these findings to a larger sample, the associations would be striking.

It is important to address the manner in which cortisol was sampled in this study. Whereas the sampling techniques were established according to sound scientific principles, and demonstrated good reliability (Stahl & Dorner,1982), the reader should note that only preliminary conclusions can be drawn from a single day of cortisol sampling. Cortisol is a labile hormone that reacts to all life experiences, as explained earlier in this paper. Thus, sampling cortisol on more than one day would provide a more stable measure of cortisol functioning, and would help to identify and account for elevations or decreases in cortisol that might be related to specific experiences or mood

changes. In addition, cortisol collection in further studies should be accompanied by some reliable indication of mood, current activity, and other factors that would inform about the participant's current state. This information would have direct relevance regarding the interpretation of cortisol patterns. Without this information, it is not possible to identify potential causes of the observed individual differences in diurnal cortisol activity, or the direction of the effect in their association with the psychosocial variables.

This study demonstrated the utility and power of multi-level modeling in analyzing nested data. HLM provided a framework within which to find meaning in these patterns of cortisol samples, exceeding the abilities of standard regression or analysis of variance. Using HLM, we were able to investigate individual differences in diurnal patterns of cortisol while incorporating information pertaining to individual cortisol samples, individual participants, and mother-child pairs.

Another element of this study that would have been of interest is an investigation into the correlation between diurnal cortisol patterns (slopes and intercepts) of mothers and their children. Unfortunately, due to limitations that arose when using the HLM software with this particular sample, we were not able to examine this question. This information would be of considerable interest for future analyses.

One of the unique elements of this study is that the samples were drawn from an ongoing longitudinal study. Longitudinal studies provide the framework within which one can observe trajectories of change, and may suggest the direction of causal relations between sequential variables. This pilot research presents the possibility of designing further prospective studies to follow the children and mothers in this sample. In doing so,

one could examine the sequelae of the effects of adverse early experiences and subsequent dysregulated diurnal cortisol patterns, in a prospective fashion.

Despite the limitations of this exploratory study, remarkably consistent results emerged. Variables such as high SES, good ability to stimulate children, and high child I.Q. were protective factors for diurnal cortisol patterns, whereas variables such as high maternal hostility and maternal smoking led to dysregulated diurnal cortisol patterns. Even when significant results did not emerge in additional predictor variables, trends always favoured the direction that could be expected, where "positive" variables had a protective effect, and "negative" ones had a detrimental effect, providing a uniform picture of results.

It is possible that these findings represent subtle individual differences in diurnal cortisol rhythms with little significance for psychosocial or physiological health.

However, it is also entirely possible that these same subtle variations are in fact markers of initial HPA dysregulation that represent vulnerabilities or risk factors towards more serious HPA dysregulation and associated mental and physiological health problems. If the latter is true, knowledge of these individual differences might provide insight into the detection of people with these vulnerabilities, and ultimately the prevention of negative health outcomes that might follow.

This study highlights the potential physiological consequences of raising children in adverse early environments that include variables such as poverty, maternal hostility, maternal smoking, and a poor ability to stimulate children, among others. As in Coplan's studies with monkeys (1996; 2001), this research suggests that the relationship between a

child and his parents, as well as the environment that surrounds his development can have physiological effects that might be long-lasting and might be damaging to a child's psychosocial and physiological well being. These findings certainly encourage more research in this domain, with an extended design to better investigate this potentially important clinical issue.

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Appendix A

"L'INDIVIDU DANS SON MILIEU: Les parents et leurs enfants"

Directeurs du projet: - Lisa A. Serbin, Ph.D.

- Dale M. Stack, Ph.D.

- Alex E. Schwartzman, Ph.D.

FORMULAIRE DE CONSENTEMENT

Je,	, m'engage volontairement avec mon enfa	nt,
parents et leur enfant" de L'étude comprend une série de mon enfant, ainsi que filmés. L'étude comporte or rémunération totale de \$50 En signe de courtoisie, le communiqués par téléphor	, à participer à l'étude "L'individu dans son milieu: L'Université Concordia. Les buts du projet m'ont été explique de questionnaires, une évaluation du fonctionnement intellecturois périodes de jeux lors desquelles nous serons observés eux sessions d'une durée maximale de 3 heures chacune et u 00 me sera allouée aussitôt que les questionnaires seront rem résultats sommaires de l'évaluation de mon enfant me sero e. De plus, les chercheurs seront prêts à effectuer une ou de besoin, pour terminer l'évaluation, discuter de résult	es és. lel et ine is. ont
Je comprends que écrites ou filmées, sont str recherche. Dans toutes les Cependant, selon la loi sur	toutes les informations que nous fournissons, qu'elles soie ctement confidentielles et qu'elles ne serviront qu'à des fins circonstances, je suis assuré(e) que l'anonymat sera conser la protection de la jeunesse, toute information indiquant de l'at re divulguée à l'Office de la Protection de la Jeunesse.	de vé.
Comme le projet "L'individêtre appelé(e) dans l'aveni	suis libre de cesser notre participation à n'importe quel mome u dans son milieu" est à long terme, je comprends que je pourr pour participer à d'autres étapes de ce projet. Je me réserve ent, de donner suite ou non à la demande de participation.	rais
Signature:		
Nom:	Date:	
Assistant(e) de recherche:		

Appendix B

PARENT-CHILD/HEALTH CANADA: Full Protocol

May 15, 1996

DAY 1 PROTOCOL:

- 1- Examiner: takes care of introductions,
 - builds rapport with child,
 - explains general Day 1 procedures to Ss,
 - makes sure mother has read and signed consent form,
 - administers HOME interview items as part of the warm-up conversation,
 - explains saliva sampling and obtains a sample from both of them immediately before standard testing (record the time that all samples are taken on the appropriate form).

- Interviewer: chooses the most appropriate room for interaction series,
 - sets up camera and materials for Series 1 in the standard order (see toy lay -out sheet).
 - removes all other unnecessary materials,
 - unplugs that room's telephone if present,
 - and attempts to remain as invisible to the child as possible until Series 2. (+20 min.).
- 2- Examiner: begins administering Bayley II or SB4.

- Interviewer: a) if mother does not need to stay with child (for SB4): Interviewer begins administering the demographic, obstetric, temperament and health questionnaires to her;
 - or b) if mother needs to stay with her child, the Interviewer can supervise siblings, do HOME observation items, score/enter data, or read a good book!!!

(30-60 min. or whatever the child can handle)

- The 2nd saliva sample is taken from both mother and child BREAK immediately (±10 min.) following standard testing. Examiner asks mother to come, if she's with Interviewer.
 - Make sure you ask Ss if they need to go to the bathroom or get a change of diaper.
 - If needed, Interviewer informs Examiner of interaction setup location.)

3- Before bringing Ss to the interaction room, the Examiner gives mother the following Series 1 instructions.

Série 1

"Maintenant, on aimerait vous voir jouer ensemble. Comme tu sais, on va enregistrer ça sur vidéo. Donc, pour être sûr que vous restiez tous(tes) les deux bien en vue pendant qu'on filme, c'est très important que vous restiez assis(es) tous(tes) les deux sur le tapis qu'on a mis par terre. Moi, je vais rester silencieuse derrière la caméra pour être bien sûr qu'elle fonctionne bien. Donc, essayez d'être le plus naturels possible et faites comme si je n'étais pas là. Alors, la première chose qu'on aimerait que tu fasses est simplement de jouer avec (ENFANT) comme vous le faites d'habitude pendant environ 15 minutes. Vous pouvez prendre n'importe quel des jouets sur le tapis. Puis, quand tu entendras l'alarme sonner, tu pourras arrêter de jouer. As-tu des questions? C'est très important aussi que tu attendes mon signal avant de commencer à jouer, OK?"

Examiner then gets Ss settled on the carpet and instructs child (if s/he can understand such instructions) to remain within its limits; e.g.:

"Maintenant, (CHILD), tu vas jouer avec maman, mais j'aimerais que tu restes sur le tapis. Fais comme si le tapis était ton carré de sable et que c'est défendu de sortir du carré de sable..." etc.

Examiner goes behind the camera and tells mother they can begin. Examiner is responsible for timing Series 1,2, and 3. The beeper should be started and stopped over the microphone so the coders are clear about when to begin and end coding that episode. [If there is an interruption of filming during the **first** half of the series (e.g., bathroom), reset the timer to 15 min. and start over. If the interruption occurs in the **second** half of the series **and** lasts less than 2 min., just pause and restart timer when the interaction resumes; but if the trip takes **more** than 2 min., Series 1 will have to be repeated at the end of Day 2.1

At the end of Series 1, Examiner administers "Maternal perceptions" questionnaire. If mother reports a score of 1 or 2, thus indicating that either her or her child's behavior was not natural, Series 1 should be repeated on Day 2.

(<u>+</u>20 min.)

BREAK - Everybody leaves interaction room during break so that the (±10 min.) Interviewer can reposition materials for Series 2, and position a barrier (e.g., Fisher Price gate, a playpen) that will <u>safely</u> prevent 12-36 mo. child from leaving interaction room during separation episode.

- Rathroom check

4- While the Examiner supervises the child away from the interaction room, she asks mother to join the Interviewer there. The Interviewer will then give mother the following Series 2 instructions so as not to be heard by child. (If child becomes upset about his/her mother's departure, Examiner will give her the instructions in the child's presence.)

Série 2 FREE PLAY (4 MIN)

"La prochaine période de jeux va aussi être filmé mais va avoir 4 parties: En premier, tu va recommencer à jouer avec (ENFANT) comme tantôt, mais juste pour une couple de minutes jusqu'à ce que tu entendes l'alarme sonner, comme tantôt."

PUZZLES (7 MIN, 4 MIN for 12-36 cohort)

"A ce moment-là, pousse les jouets de côté et choisis un casse-tête à faire avec (ENFANT). (FOR OLDER COHORT, EXPLAIN TO MOTHER THE LABELLED BAGS OF PUZZLE PIECES AND THEIR CORRESPONDING BOARDS). Si vous finissez ce casse-tête-là, vous pouvez travailler sur un autre. Après quelques minutes, l'alarme va sonner de nouveau et je vais entrer pour m'asseoir ici." (PRESS BEEPER WHEN THEY BEGIN WORKING ON THE PUZZLE)

Interviewer comes in at the beep and waits next to the door until mother has left. Then s/he puts the barrier in place (for 12-36 mo. cohort) and sits down on a chair so as not to face child directly. Interviewer then gets busy with paperwork interacting as little as possible with child (i.e., s/he should not look at, speak to, or touch the child unless s/he is in danger of harming him/herself).

SEPARATION AND REUNION (2+4=6 MIN)

"A ce moment-là, tu sortiras de la pièce pour laisser (ENFANT) jouer tout seul avec les jouets. Et pour être sûr qu'il/elle ne te suivra pas quand tu va sortir, je vais placer une barrière en travers la porte/arche. Bien sûr, si (ENFANT) devient trop dérangé par ton absence, ou si tu te sens mal à l'aise, on arrêtera puis tu pourras le/la rejoindre. Sinon, après une couple de minutes, je vais sortir pour te dire que c'est le temps d'aller rejoindre (ENFANT) sur le tapis. Puis, tu passera 3-4 minutes de plus avec lui/elle et on te laissera savoir quand tout est fini."

Examiner programs beeper for 6 min. and presses "start" when mother exits the room. Then, after 2 minutes, she signals Interviewer to go get mother by pressing "pause" and presses "start" again when mother comes in. Examiner should keep child in view during separation and reunion episodes.

"Donc, pour résumer, commencez par jouer ensemble comme vous le faites d'habitude; puis, quand tu entendras l'alarme, pousse les jouets de côté et choisis un cassetête. Quand tu me verras entrer, sors de la pièce jusqu'à ce que je te dise te rejoindre (ENFANT). J'ai une petite liste qui pourra t'aider à te souvenir des étapes, et je vais la placer juste ici. As-tu des questions? J'aimerais juste te rappeler encore de rester sur la

couverture pour que vous puissiez rester bien en vue. J'aimerais aussi quand tu sortiras que tu restes invisible pour (ENFANT), mais assez près pour entendre l'alarme. N'oublie pas d'attendre le signal avant de commencer, OK?"

At the end of Series 2, Interviewer takes cortisol sampling and then administers "Maternal perceptions" questionnaire If mother reports a score of 1 or 2, Series 2 should be repeated on Day 2. The interviewer then takes the final saliva sample from both the parent and her child.

(+25 min.)

5- At the end of Day 1, Interviewer administers Day 1 Touch Questionnaire, gives instructions for mother and father questionnaire packages, and summarizes Day 2 procedures.

N.B. If child needs to nap during Day 1, Interviewer can take that opportunity to begin interviews with mother.

Total time, 2-3 hours

Fill out the VideoTape log sheet. Clean Bayley II and toys between each visit

DAY 2 PROTOCOL:

1- Examiner reconnects with child. Rapport building between Interviewer and mother, this includes Day 2 general instructions.

(+15 min)

2- Examiner finishes Bayley II or SB4. If mother does not need to stay with child, Interviewer answers any questions she might have about the questionnaires and finishes interviewing her. But if mother still needs to stay with child, Interviewer can set up Series 3 materials.

BREAK - Series 3 setup, if not done already (+10 min.)

- Bathroom check
- 3- While Examiner supervises child away from interaction room, she tells mother to go to the interaction room to meet Interviewer who gives her the following Series 3 instructions so as not to be heard by child. If child becomes upset about mother's departure, the Examiner gives her the instructions in the child's presence.

Série 3 FREE PLAY (4 MIN) "C'est la dernière fois qu'on va vous filmer, et il y a 4 choses qu'on aimerait que vous fassiez ensemble. D'abord, comme l'autre jour, on aimerais que tu joues avec (ENFANT) comme vous le faites d'habitude avec les jouets jusqu'à ce que tu entendes l'alarme sonner.

COMMAND TASK (3 MIN) NOT DONE FOR 12-24 MO. CHILDREN

A ce moment-là, vous aller arrêter de jouer pour faire quelque chose de complètement différent. Pour les 2-3 prochaines minutes, j'aimerais que tu demandes à (ENFANT) de faire quelques petites tâches pour toi. Tiens, voilà une liste de tâches que tu peux utiliser (GIVE HER THE PAD). Comme tu peux voir, il y en a qui sont plus difficiles que d'autres; c'est parce qu'on visite différentes familles avec des enfants d'âges différents. Celles du début sont plus faciles que celles de la fin (READ FIRST 3 AND LAST 3). On aimerais que tu prennes au moins 4 ou 5 des tâches de la liste. Tu peux en prendre plus si tu veux et tu peux même inventer tes propres tâches, mais pourvu que (ENFANT) n'ait pas à sortir de la pièce. Le pad sera placé tout près du tapis. (PRESS BEEPER WHEN MOTHER BEGINS INTRODUCING TASK)

INTERFERENCE TASK (3 MIN)

Quand tu entendras l'alarme sonner, vous arrêterez pour faire autre chose encore. On aimerais voir comment (ENFANT) réagit quand tu es très occupée. Tu sais comment c'est des fois quand tu es au téléphone ou bien en train de faire à manger et que c'est pas possible de lui donner toutes l'attention qu'il/elle demande. Pour observer ça, on aimerais que tu tournes la page sur ton pad pour remplir les questionnaires qui sont juste en-dessous (SHOW HER). Et pendant que tu les remplis, on aimerait que tu te retournes un peu pour lui faire comprendre que ce que tu fais est très important. (ENFANT) pourra continuer à jouer avec les jouets pendant ce temps-là; mais assure-toi encore qu'il/elle reste assis(e) sur le tapis. Tu continueras de travailler sur les questionnaires jusqu'à ce que tu entendes une autre alarme. (PRESS BEEPER WHEN MOTHER BEGINS QUESTIONNAIRE)

FREE PLAY (4 MIN)

A ce moment-là, mets le pad de côté et recommence à jouer avec (ENFANT) comme vous le faites d'habitude jusqu'à ce l'alarme te dise que c'est fini. N'oublie pas de rester à l'intérieur des limites du tapis pour que la caméra puisse vous garder tous les deux bien en vue.

Donc, en résumé, commencez par jouer avec (ENFANT) comme vous le faites d'habitude; ensuite, quand tu entends la lère alarme, prends le pad et fais-lui faire des tâches; puis, à la 2e alarme, commence à travailler sur le questionnaire jusqu'à ce que tu entendes la 3e alarme. A ce moment-là, tu recommences simplement à jouer avec (ENFANT). Comme la dernière fois, on a une petite liste qui va t'aider à te rappeler des étapes. As-tu des questions? N'oublie pas d'attendre le signal avant de commencer, OK?"

At the end of Series 3, Interviewer administers "Maternal perceptions" and "Touch" questionnaires.

 $(\pm 25 \text{ min.})$

BREAK +10 min.

4- Examiner administers the "Parenting Practices Interview", investigate any clinical concerns that might have arisen through other questionnaires, administers the remaining HOME interview items and the SCID modules (if required). Meanwhile, the Interviewer administers the Peabody to the child. When Examiner is done with her interviews, the Interviewer joins her for the wrapup.

(±60 min. or more, as needed)

Total time, 2-3 hours.

Fill out the VideoTape log sheet. Clean Bayley II and toys between each visit.

Appendix C

ÉCHANTILLONS DE CORTISOL

RÉVISÉ 96-05

- "Parmi les choses qu'on va faire pendant la session d'aujourd'hui, on va prendre des échantillons de salive, avec toi et avec ton enfant. Tu vas voir c'est très simple et on en prend seulement trois de toute façon." Si la participante a déjà participé aux tests sur le stress, elle sait déjà comment faire.
- Si <u>rouge ou protecteur à lèvres</u>: le faire enlever car le gras qu'il contient nuit à ce qu'on regarde dans la salive.

FAIRE LA DÉMONSTRATION SOI-MEME,

- "On va en prendre un tout de suite. Voici comment on fait: Tu prends la languette par le bout plié seulement. Il ne faut pas que tu touches au bout qui va dans ta bouche avec tes doigts ou ton menton, à cause du gras qu'on a sur la peau; ça dérangerait l'échantillon. Tu mets la languette sur ta langue ou, si tu préfères, en-dessous de ta langue. Sur la langue, ça va souvent mieux et il y a moins de chance de briser le papier. Tu attends un peu pour que le papier soit complètement imbibé de salive, jusqu'au pli, avant de le remettre dans l'assiette."

Montrer la languette mouillée et dire au S:

- "OK, c'est à ton tour maintenant... Si tu sens que tu manques de salive, attends de t'en faire suffisamment avant de mettre le filtre sur la langue. Malheureusement, tu ne peux pas boire de l'eau juste avant, car ça va diluer ta salive. Mais tu pourrais penser que tu viens de prendre une bonne mordée dans un citron ou une limette. C'est un assez bon truc pour provoquer la salive."

A PROPOS DES ÉCHANTILLONS DE SALIVE PRIS À LA MAISON

- "Je vais aussi te laisser des languettes pour prendre des échantillons de ta salive et de celle de (ENFANT) comme tu l'as fait ici aujourd'hui. On t'en donne 24, 12 languettes pour toi et 12 autres pour (ENFANT). Elles sont numérotées de M-I à M-I2 pour toi, la mère, et de E-I à E-I2 pour ton enfant. Tu vas prendre un échantillon de ta salive et un échantillon de celle de (ENFANT) toutes les 2 heures, pendant que vous êtes réveillé(e)s. C'est-à-dire du moment où chacun de vous se lève jusqu'au coucher."

À propos de l'horaire à la maison: [Lui montrer le compte rendu.]

- "Il y a une page à remplir pour toi et le verso est pour (ENFANT). Tu vas voir, c'est pas long à faire. La première chose à faire, c'est d'écrire la date. Ce qui est très important, c'est que tu prennes ta salive aux heures qu'on te demande, c'est-à-dire aux heures impaires: 7, 9, 11... en plus de l'échantillon au lever et de celui au coucher. La première prise de la journée, c'est très important et tu vas la prendre tout de suite en te levant, quelle que soit l'heure à laquelle tu te lèves. Après ça, tu vas suivre l'horaire. Par exemple, si tu te lèves à 9h30, tu prends ta première salive à 9h30 mais tu reviens ensuite à l'horaire demandé même s'il n'y aura pas 2 heures entre ta première et ta deuxième prise. Donc, ta deuxième prise serait à 11h00 dans ce cas-ci."

[Parler du reste de l'horaire (heures impaires, écrire l'heure des repas...) y compris l'échantillon de l'heure du coucher qui n'est pas nécessairement prise 2 heures après la dernière heure impaire.]

Lui faire voir la page derrière le compte rendu:

[Mentionner <u>notre # de tél. et lire</u> cette page au S en expliquant un peu plus et en lui disant de relire cette page avant de commencer.]

"Une chose qui est vraiment très importante pour nous, c'est qu'on a besoin d'échantillons à l'heure où vous vous levez parce que c'est là que le cortisol est à son plus haut dans le corps. On veut donc savoir d'où chaque personne part. Pour ne pas oublier le matin où tu commences, mets les langettes à l'endroit le mieux pour toi pour ne pas oublier... comme ta table de nuit..." [En parler avec le S.] "Tu dois écrire l'heure exacte à laquelle tu as pris chaque échantillon, même si ce n'est pas l'heure demandée. Écris aussi le numéro des languettes, en suivant l'ordre: 501 M-1, 501 M-2... On aimerait aussi que tu écrives dans la case appropriée comment toi ou ton enfant se sentait au moment de l'échantillon de salive. (Les situations doivent être stressantes pour le participant, et indiquer aussi quand ça va bien. On veut une remarque pertinente à chaque prise de cortisol.) Par exemple, si tu as mangé lors de la deuxième prise de cortisol, on veut savoir comment tu te sentais en mangeant et non pas seulement que tu as mangé."

- Montrer comment remplir le bas de chaque page du compte rendu.

"Il est aussi bien important de faire sécher les languettes à <u>l'air libre</u>, à la température de la pièce (<u>pas</u> dans un four ordinaire, <u>ni</u> un four à micro-ondes, <u>ni</u> un séchoir à cheveux, etc.), avant de nous les retourner. Donc, chaque fois que tu prends ta salive, il faut que tu laisses la languette à l'air pendant environ 8 heures pour qu'elle soit complètement sèche. Tu peux la mettre dans une assiette.

- [Discuter avec S d'un bon endroit où il/elle peut laisser sécher les languettes à la maison.]

"Si tu ne peux pas la laisser sécher tout de suite après l'avoir prise (par exemple, si tu travailles sur la route...), remets la languette dans son petit sac en plastique et fais-les toutes sécher en même temps en arrivant à la maison. Pour ça, coupe le petit sac de chaque côté, en faisant bien attention de ne pas couper la languette. Ensuite ouvre le sac et laisse ça sécher. Cependant, tu dois faire bien attention à 2 choses:

- 1) Chaque <u>languette mouillée</u> doit être <u>toute seule dans son petit sac de plastique</u> (donc, une seule languette par sac).
- 2) Fais aussi attention pour toujours <u>prendre la languette par le bout plié</u> et ne jamais y toucher par la partie qui va dans la bouche.

Quand la languette est complètement séchée, la mettre dans le plus grand sac de plastique approprié, les tiennes dans le sac marqué 'Mère' et celles ton enfant dans le sac marqué 'Enfant'. On ramassera le tout lorsqu'on reviendra la semaine prochaine."

Appendix D- Demographic Information Questionnaire

Septembre 1996			N° d'identification Date:
	L'INDIVIDU DA Renseignements so	NS SON MILIEU ciodémographiques	J
	Tous ces renseignements s	ont traités de façon totale	ment confidentielle
1.Sexe ☐ M 2.Âge		AN MO JR	
3.État civil			
mariée	oints de fait": désigne deux per es. Il s'agit de ton état actuel; m vis avec un(e) conjoint(e) prés	ême si tu es légalement divor	rcé(e) ou autre, mais
_	☐ Célibataire	☐ Conjoint	Depuis quelle date?
	☐ Marié(e)	☐ Séparé(e)	AN MO JR
	☐ Divorcé(e)	☐ Veuf/veuve	
4. Nombre d	l'enfants		
Si enceint	e (ou conjointe enceinte). bé		AN MO
Sinon, pré	voyez-vous avoir un enfant		NON
	,	dans les prochains 24 mois	? OUI NON

Pour chaque enfant:

- 1 Inscrire le nom, le sexe. la date de naissance
- 2 Encercler "TE" si c'est ton enfant (tu es le parent biologique)
 "EC" si l'enfant du conjoint (le conjoint actuel est le parent

"EC" si l'enfant du conjoint (le conjoint actuel est le parent biologique)

"EA" si c'est un enfant adopté /"FA" en foyer d'accueil et qui vit chez

toi

Si "TE" et "EC" sont vrais, encercler les deux.

3 - Indiquer si l'enfant vit avec toi	i, OUI ou NON ou GP (garde partagée)
ou une école spéciale.	plicable) ainsi que si l'enfant fréquente une classe
1 NOM	SEXE AN MO JR
	🗆 м 🗆 ғ
L'enfant est: TE EC EA/F.	A Vit avec toi: OUI NON GP
Année scolaire:	_ Classe spéciale:
2 NOM	SEXE AN MO JR
	ПМ П Г
L'enfant est: TE EC EA/F	A Vit avec toi: OUI NON GP
Année scolaire:	Classe spéciale:
	- -
3 NOM	SEXE AN MO JR
	🗆 м 🗆 ғ
L'enfant est: TE EC EA/F	FA Vit avec toi: OUI NON GP
Année scolaire:	Classe spéciale:
	-
4 NOM	SEXE AN MO JR
	□м □ F
L'enfant est: TE EC EA/E	FA Vit avec toi: OUI NON GP
Année scolaire:	Classe spéciale:
	-
5. Ta scolarité complétée (des	
En quoi? (spécialisation/général)):

Étudies-tu présentement? OUI : Temps plein Si oui, quel diplôme postules-tu	
6. As-tu un emploi (rappel: renseignements gardés co	onfidentiels)?
,	NON □
Occupation:	As-tu déjà eu un emploi?
Tes tâches:	Oui □ Non □ ↓ En quoi?
	Pendant combien de temps? an(s) mois
Salaire de l'heure\$	Quand as-tu arrêté de
travailler: Depuis quand es-tu à cet emploi? inscrire la date AN MO //	date:// AN MO
Au cours des 12 derniers mois, as-tu bénéficié de:	
Oui ☐ Non ☐ l'Assurance chômage?	
Oui Non Prestations d'aide sociale?	
Oui Non la CSST? (préciser:	
7. Informations sur le conjoint (renseignements g AN MO JR	ardés confidentiels):
a) Son nom:b) Date de naissance	<u> </u>
Son occupation:	
Sec tâchec	

	Son salaire:	\$/ heure	4 3.7	\ (0	Nombre d'heu	res	_/ semaine
	II/Elle travaille là dep	puis: date	AN —				
b)	Au cours des 12 dern	iers mois,	a-t-il/e	elle bé	néficié de:		
	Oui 🛘 Non 🗖	l'Assuran	ce chá	òmage	?		
	Oui 🗆 Non 🗆	Prestation	ns d'ai	de soc	iale?		
	Oui 🗆 Non 🗖	la CSST	? (préc	iser:_)
c)	Sa scolarité compléte	<u>ée</u> (dernière	e anné	e term	inée):		
	En quoi? (spécialisat	tion/généra	I):			-	
	Étudie-t-il (elle) prés	sentement?	OUI	: Tem	ps plein 🛭 pa	rtiel 🗆	NON □
	Si oui, diplôme posti (date)/	ulé?			pour q	uand?	
8.	Informations sur le	père\la m AN M			nfants (si n'hab	oite pas a	vec toi)
a)	Son nom:						
b)	Date de naissance			_			
	Son occupation:						
	Ses tâches:						
	Son salaire:	_\$/ heure			Nombre d'h	eures	/ semaine
	Il/Elle travaille là de	puis: date		MO 			
b)	Au cours des 12 der	niers mois,	a-t-il/	elle b	énéficié de:		
	Oui 🗆 Non 🗖	l'Assura	nce ch	ômag	e?		
	Oui 🗆 Non 🗖	Prestatio	ons d'a	ide so	ciale?		
	Oui 🗆 Non 🗀	la CSST	? (pré	ciser:_)
c)	Sa scolarité complét	<u>tée</u> (demièi	re anno	ée terr	ninée):		
	En quoi? (spécialisa	ition/généra	al):			_	
	Étudie-t-il (elle) pré	sentement	? OU	i : Ten	nps plein 🔲 pa	artiel 🗆	NON □

Si oui, diplôme postulé?		pour quand? (date)/	pour quand? (date)/		
S.V.P. Vérifier l'a	dresse et les numéros de t	éléphone.			
No	Rue	app.			
Ville		Code postal			
Téléphones:		_) _)			
Autre	: (_)			
Ton numéro de té lien avec toi:	léphone est à quel nom da	ans l'annuaire téléphonique: Nom complet	t et		
Adresse électroni	que:				
Adresse des pare	nts:				
	· · · · · · · · · · · · · · · · · · ·				
					

Appendix E PUPIL EVALUATION INVENTORY

AGGRESSION ITEMS

- 3. Those who can't sit still.
- 4. Those who try to get other people into trouble
- 7. Those who act stuck-up and think they are better than everyone else.
- 8. Those who play the clown and get others to laugh.
- 9. Those who start a fight over nothing.
- 12. Those who tell other children what to do.
- 15. Those who always mess around and get into trouble.
- 16. Those who make fun of people.
- 18. Those who do strange things.
- 20. Those who bother people when they're trying to work.
- 21. Those who get mad when they don't get their way.
- 22. Those who don't pay attention to the teacher.
- 23. Those who are rude to the teacher.
- 26. Those who act like a baby.
- 27. Those who are mean and cruel to other children.
- 29. Those who give dirty looks.
- 30. Those who want to show off in front of the class.
- 31. Those who say they can beat everbody up.
- 33. Those who exaggerate and make up stories.
- 34. Those who complain nothing seems to make them happy.

WITHDRAWAL ITEMS

- 5. Those who are too shy to make friends easily.
- 6. Those whose feelings are too easily hurt. (Not used)
- 10. Those who never seem to be having a good time.
- 11. Those who are upset when called on to answer questions in class. (Not used)
- 13. Those who are usually chosen last to join in group activities.
- 17. Those who have very few friends.
- 24. Those who are unhappy or sad.
- 28. Those who often don't want to play.
- 32. Those who aren't noticed much.

LIKEABILITY ITEMS

- 2. Those who help others.
- 14. Those who are liked by everyone.
- 19. Those who are your best friends.
- 25. Those who are especially nice.
- 35. Those who always seem to understand things